More ribosomal spacer sequences from Xenopus laevis

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ABSTRACT

The base sequence analysis of a Xenopus laevis ribosomal DNA repeat (7) has been extended to cover almost the entire non-transcribed and external transcribed spacer. A compilation of these sequences is presented. All the repetitive and non-repetitive sequence elements of the spacer are identified and their evolution discussed. Comparison of the X.laevis and S.cerevisiae (25,26) ribosomal DNAs shows about 80% sequence conservation in the 18S gene but no sequence conservation, from the available data, in the external transcribed spacer. The sequence coding for the 3' terminus of the X.laevis 40S ribosomal precursor RNA is presented and its structural features analyzed.

INTRODUCTION

The repetitive ribosomal genes of eukaryotes are clustered at the nucleolar organizer, commonly equated with the secondary constriction observed in metaphase chromosomes. During the cell cycle the chromatin of the nucleolar organizer undergoes dramatic structural changes. In certain hetero-species hybrids the appearance of a secondary constriction is suppressed in one chromosome set (1), while in other such hybrids the secondary constriction is seen but no nucleoli are organized (2) and only one set of ribosomal genes is activated in early development (3). Quite possibly, the ribosomal spacer DNA may play a role in these suppression mechanisms since this DNA is known to differ extensively between species, unlike the ribosomal RNA coding sequences which are highly conserved.

In the past the non-transcribed ribosomal spacer DNA was considered to be biologically inactive. Recently however, it has been shown that replication of ribosomal DNA (rDNA) initiates in the non-transcribed spacer of Drosophila melanogaster (4,5). Furthermore, a regulatory phosphoprotein which binds to the rDNA spacer of Physarum polycephalum has recently been
discovered (6).

In order to understand the molecular basis of the structural and functional properties of the ribosomal gene and its spacer it may be essential to know the primary structure of this DNA. Our previously published sequence analysis has revealed that the spacer DNA of a cloned rDNA of Xenopus laevis includes a variety of repetitive and non-repetitive elements interspersed with one another (7). Most strikingly, however, we discovered that the putative promoter sequence lying immediately upstream of the 5' end of the 40S pre-rRNA coding sequence has, in evolutionary terms, been reduplicated (8) and can now be found in nearly unmutated form as the so-called Bam Islands transposed into spacer DNA. In the related species Xenopus borealis, a similar situation obtains in that a 40 bp sequence bridging the 5' terminus of the 40S coding sequence is seen to be repeated several times in the spacer DNA (9). Embedded in this repeated DNA element there is a short sequence which is held in common between the two species (9) pointing to a highly conserved and important function such as initiation of transcription. Whether the reduplication of the Bam Island-5' sequence motif is of biological significance remains to be established.

Using the poly(dA) tailing technique for rapid ordering of DNA fragments (8,10) and DNA sequencing we have now an appreciation of almost the entire rDNA gene spacer sequence (7), this paper and unpublished results) stretching from the 28S to the 18S coding sequences with the principle exception of the coding sequence at the 5' end of the 18S gene and of the repetitive region 3 which is virtually identical to repetitive region 2 in its restriction pattern (7) and hence does not warrant a special investigation. We present a sequence compilation and make some additional comments on the evolution of spacer DNA. We also discuss some interesting structural features of the region coding for the 3' end of the 28S RNA which is thought to contain the termination signal for transcription.

MATERIALS AND METHODS

Restriction enzymes were obtained from New England Bio Labs, terminal transferase from Boehringer Mannheim, bacterial alkaline phosphatase from Worthington Biochemical Co. and T4 poly-nucleotide kinase from P-L Biochemicals. λ exonuclease, prepared essentially as described by Radding (11), was a gift of Drs. H.O. Smith and S.G. Clarkson. Unlabelled dATP was obtained from Sigma Chemical Co. (3H)-dATP (25Ci/mmol) and (32P)-ATP (3000Ci/
mmol) from Amersham-Searle. Poly (dT) cellulose was grade T3 from Collaborative Research Inc. All other reagents were of analytical grade.

Poly(dA) tailing

The 6.6 kb rDNA insert of pX1108 was excised and purified by CsCl-actinomycin centrifugation as described previously (12). Endonuclease cleavage was carried out under conditions recommended by the enzyme supplier. 0.5 mg of the 6.6 kb fragment was incubated at 0.11 mg/ml with 600 units/ml λ-exonuclease (λ-Exo) in 67 mM glycine-KOH pH 9.4, 2.5 mM MgCl₂ (13) at 14°C. Digestion was terminated after 2 1/2 min, in the case of the intact 6.6 kb fragment, or after 6 min, in the case of the Bam HI restricted fragment, by addition of EDTA to 10 mM and NaCl to 0.3 M, both on ice. The solution was then extracted twice with phenol-chloroform (1:1, v/v), twice with chloroform-isooamylalcohol (24:1, v/v) and the DNA ethanol precipitated. The λ-Exo treated DNA was incubated at 1.2 mg/ml with 730 units/ml (-v0.1 mg/ml) terminal transferase in 100 mM Na cacodylate, pH 7.0, 8 mM MgCl₂, 1 mM dATP (14,15), 60 μCl/ml (³²P)-dATP for 30-40 min at 37°C. The reaction was terminated and the DNA recovered exactly as described following λ-Exo treatment.

Partial restriction and poly(dT) cellulose chromatography

Partial restriction was allowed to proceed until only a third of the initial DNA fragment was left intact. This extent of restriction was calculated to give the maximum yield of the larger restriction products (10). Restriction was terminated by addition of EDTA to 10 mM, the solution was diluted five times with 0.4 M NaCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA and applied to a 0.4 ml column of poly(dT) cellulose equilibrated in the same buffer (16). The column was then washed with several millilitres of application buffer and the poly(dA) tailed fragments eluted with one bed volume of 10 mM Tris, 1 mM EDTA, pH 8.0.

DNA sequence analyses were performed according to Maxam and Gilbert (17) with the later modifications (18).

Strand separation after poly(dA) tailing

The DNA in 10 mM Tris-HCl, 1 mM EDTA pH 8.0 and at a concentration low enough to prevent rapid renaturation was heated to 100°C for 5 min in a siliconized Eppendorf tube, then cooled on ice. NaCl was added to a final concentration of 0.4 M, and the solution applied to a 0.4 ml poly(dT) cellulose column, as described above. The column was washed and eluted as above except that the eluant was collected and further handled in siliconized equipment.
RESULTS AND DISCUSSION

Strategy of sequence analysis

The poly(dA) tailing-partial restriction technique described previously (8,10) was adopted to facilitate the sequence analysis of the Xenopus laevis ribosomal spacer DNA contained in the recombinant plasmid pXL108 (7). The technique, which has also been applied to transcript mapping (8), has several distinct advantages over the more classical methods of fragment preparation generally employed for sequence analyses: a) the fragments obtained overlap each other and thus sequence overlap is usually also obtained; b) fragments are automatically ordered by their size and are usually separable by gel electrophoresis. This is also the case when the DNA contains highly repetitive elements and c) secondary restriction or strand separation to obtain unique labelled fragments is usually unnecessary. By way of illustrating the technique, the strategy of sequence analysis of a repetitive spacer region is described in detail below.

The 6.6 kb rDNA fragment, obtained from pXL108 by restriction with EcoRI and subsequent separation on a CsCl actinomycin gradient (12) was poly(dA) tailed at its 3' termini using terminal (deoxynucleotide) transferase and then restricted with Bam H1 (Fig.1a). The resultant fragments were separated by preparative gel electrophoresis (19) and the 2.2 kb left hand fragment subjected to partial restriction with Hinf I. The poly(dA) tailed fragments were separated from the untailed fragments by affinity chromatography on poly(dT) cellulose, 5' 32P labelled using polynucleotide kinase (PNK) (20) and fractionated by gel electrophoresis. The purity of the fragments recovered after electrophoresis is demonstrated in Fig.1c. These fragments were subjected to DNA sequence analysis (17).

In a second experiment (see Fig.2a) the 6.6 kb rDNA fragment was restricted with Bam H1 and then the mixture of fragments poly(dA) tailed with transferase before separating them by gel electrophoresis. The left hand fragment (Fig.2a) now poly(dA) tailed at its EcoRI and Bam HI termini, was partially restricted with Hinf I, the poly(dA) tailed fragments selected on poly(dT) cellulose and these DNA fragments 5' 32P labelled using PNK (20). The 5' labelled poly(dA) tailed fragments were restricted to completion with Alu I (Fig.2a), and again passed over a poly(dT) cellulose column. By this
Fig. 1. The basic poly(dA) tailing technique as applied, (a) to the repetitive region I and (b) to the external transcribed spacer of the X. laevis rDNA fragment of pXl 108. The electrophoretic fractionation of the fragments prepared in experiment (a) is shown in (c) where the gel tracks show (1) the poly(dA) tailed fragments before Hind III cleavage, (2) the same fragments after Hind III cleavage and (3) to (11) the fragments obtained after preparative fractionation of (2). Stars indicate $^{32}$P 5' label.

procedure, fragments originally terminating at the EcoRI site and now un-tailed were separated from those terminating at the Bam HI site and thus still poly(dA) tailed. The now separate populations of poly(dA) tailed and un-
Fig. 2. An extension of the basic technique as applied to (a) the repetitive region 1 and (b) the repetitive region 2 of the X.laevis rDNA. The electrophoretic fractionation of the fragments from experiment (a) is shown in (c) where the gel tracks show: (1) the untailed fragments originally terminating at the left hand EcoRI site, (2) the mixture of tailed and untailed fragments i.e. of tracks (1) and (3), before the second poly(dT) fractionation, see text, (3) the poly(dA) tailed fragments terminating at the Bam HI site, (4) to (11) fractionation of the fragments shown in (3) and (12) PM2 DNA restricted with Hae III (39). Stars indicate $^{32}$P 5' label

tailed fragments were fractionated by gel electrophoresis (Fig.2c) and each fragment sequenced directly. In this way sequence data was obtained from each Hinf I restriction site in both directions, i.e. towards the EcoRI site using the untailed fragments and towards the Bam HI site using the poly(dA) tailed fragments.
Fig. 3a and b show some examples of the sequencing gels obtained using the fragments from the two above experiments. That the fragments were pure and asymmetrically \(^{32}\text{P}\)-labelled is clearly seen from the comparison of "G"-tracks (Fig. 3a). It should be particularly noted that in the case of the poly(dA) tailed fragments (see Fig. 3b) no \(^{32}\text{P}\)-label is detected adjacent to the poly(dA) tail. The 3' poly(dA) tail obviously strongly inhibits \(^{32}\text{P}\)-labelling of the 5' terminus of the adjacent DNA strand.

If the poly(dA) tailed fragments are denatured before 5' labelling (17), as might be necessary after partial restriction with enzymes producing "flush ends" or "3' overhangs", the untailed strand will clearly also be labelled. However, in such a situation it is possible to separate the poly(dA) tailed strand from the untailed strand on a poly(dT) cellulose column and thus to facilitate sequence analysis (see Materials and Methods). This approach has recently been employed to "strand-separate" DNA molecules on a preparative scale (T. Moss, unpublished results).

The poly(dA) technique was also applied in two further experiments described in Fig. 1b and 2b. Further examples of the sequencing gels obtained are given in Fig. 3c and d, where the sequence overlap obtained when consecutive partial restriction fragments were analyzed is clearly demonstrated.

Combined base sequence data for the rDNA fragment of pX1108.

The sequence data obtained for the Xenopus laevis rDNA clone pX1108 have been combined with that previously published (7). The combined sequences, shown in Fig. 4, have also been aligned to indicate the various repetitive and non-repetitive regions of the ribosomal spacer DNA. The newly presented sequences cover the repetitive regions 0, 1 and 2 and the region coding for the putative 3' terminus of the 40S pre-rRNA transcript. The derivation of these sequences is shown at the foot of Fig. 4.

Since the X.laevis ribosomal spacer DNA is extremely GC-rich and often palindromic in sequence, some difficulties were encountered in its analysis. While very often attempts were made to sequence both strands of the DNA, this was not always possible. For example, overlapping sequences could be obtained from the Hinf I sites of repetitive region 1 when sequencing the "lower" or "coding" DNA strand. But when sequencing the complementary strand, renaturation artefacts often prevented reading of the sequencing gels past nucleotide 40. We have taken account of these inherent difficulties by indicating in Fig. 4 which sequences were determined on both DNA strands and which on
Fig. 3. a) Comparison of the "G" specific cleavage ladders (17) of repetitive region 1 fragments prepared in the experiment of Fig.2a. The tracks (1) to (8) were obtained using respectively the fragments of tracks (3) to (10) of Fig. 2c. b) An example of the sequence analyses of poly(dA) tailed fragments, (Fig.2c, track 4). Note, as judged from the clarity of the analysis, no contaminating 5' 32p label is detectable adjacent to the 3' poly(dA) tail. c) and d) Sequence analyses of Ava I partial restriction fragments respectively of repetitive region 2 (Fig.2b) and the external transcribed spacer (Fig.1b). The arrows indicate the positions of the next Ava I sites from which overlapping sequence was obtained. The bands seen in all tracks at the positions of the arrows are due to the very limited single strand cleavage activity of Ava I. A similar observation was made in the experiments using Hinf I.
Fig. 4. The compiled sequence data of the X.laevis rDNA spacer fragment of pXI 108 (7). The data is a compilation of that previously published (7) and that presented here. The extent of homologies between the Bam Islands and the 40S 5' terminal region are indicated by underlining, the transcribed regions are indicated by shading. Derivations of the sequences presented here are shown as line-diagrams where the arrows indicate the origin, direction and length of each sequence obtained. Uncertain bases are indicated by a heavy dot and sequences determined on both strands by a fine broken line.

one strand only, although the latter have usually been determined several times, and have denoted uncertain bases by a heavy dot.

It should be noted that the previously published sequences (7,8) have been thoroughly rechecked and where necessary updated in the light of new data. Notably, minor changes have been made to the previously published sequences (7,8) in the regions of the Bam Islands 1 and 2, a short unknown sequence and uncertain base near position 4370 (7) determined and a related single base error corrected. The restriction map predicted by the sequences of Fig.4 agrees astonishingly well within experimental error with that obtained by restriction (7) for all enzymes studied, with the exception of a single Hae II/Hha I site near position 1350 (Fig.4) which is not found in the sequence. We have attempted to resolve this disagreement but have not yet been successful.
Nucleic Acids Research

Sequence organization in the Xenopus laevis rDNA spacer

Recent work from this laboratory (7) has identified the major regions of X.laevis ribosomal spacer DNA. The extensive sequence data presented here however allows a more detailed discussion of the organization and evolution of the spacer DNA.

Firstly, we identify, in addition to the regions previously defined (7), a minor repetitive region which, in line with the previous nomenclature (7) we refer to as repetitive region 0. We also conclude that no further highly repetitive regions exist within the spacer DNA (Fig.4 and T. Moss, unpublished data).

Repetitive regions 0 and 1. Beginning with the 28S coding sequence and moving across the putative 3' terminus of the ribosomal transcriptional unit into the non-transcribed spacer (NTS), one arrives first at a DNA extremely rich in GC and palindromic in nature, which will be discussed in detail below. Preliminary data (unpublished results) suggest that this sequence pattern continues up to the point at which repetitive region 0 (rep.reg.0) starts. Rep.reg.0 consists of three complete and nearly identical 34 bp units. It is separated from rep.reg.1 by a 90 bp sequence which contains short homologies to both rep.reg.0 and 1. Rep.reg.1 consists of a 100 bp unit repeated slightly more than seven times (see Fig.4), the central five of these seven units being almost perfectly homologous. However, on both extremities of this region, perfect homology is broken by base exchanges, insertions and deletions such that the transition to the semi-homologous flanking sequences is ill-defined. Both the 5' and 3' flanking sequences of rep.reg.1 contain oligo-dA tracts, a very unusual feature for this ribosomal spacer DNA. Furthermore, two closely spaced oligo(dG) tracts, the longest in the spacer, occur close to the 5' side of the rep.reg.0 (data not presented).

Making use of the restriction mapping data of Botchan et al. (21) which may now be interpreted in terms of the regions shown in Fig.4, it is clear that the number of repeat units in region 0 and 1 varies in different X.laevis ribosomal spacers. From these authors' data, rep.reg.0 of clone Xlr13 contains three repeat units, i.e. similar to the clone studied here, while clone Xlr12 has five repeat units and clone Xlr14 as many as nine. In contrast, rep.reg.1 varies little between the various clones studied, containing between 6 and 8 repeat units. The variability in the number of repeat units making
up these regions could have easily been the result of unequal crossing over during recombination.

The Bam Islands and repetitive regions 2 and 3. Moving further downstream through the spacer one arrives at the Bam Island sequences which bound rep. reg. 2 and 3. These units together form the so-called "super repeats" which are seen in various multiplicities in all X.laevis rDNA spacers so far studied (7,21).

For convenience we define here the term Bam Island (7) as the region of homology between the sequences surrounding the Bam HI restriction sites. The 5' boundaries of the Bam Islands 1 and 2 then lie respectively at sequence positions 2097 and 3212 as indicated in Fig.4. From our sequence data it is not at present possible to accurately define their 3' boundaries. However restriction data (7) indicate that the Bam Island 1 most probably terminates near position 2347, i.e. at the beginning of rep. reg. 2, and sequence data (22) support this conclusion (see Fig.5).

We have previously shown that the Bam Islands contain a 145 bp sequence also found immediately upstream of the 40S pre-rRNA initiation site and thus are at least in part derived from an initiation site duplication event (7,3). Fig.5 shows the sequence homologies between the Bam Islands and initiation site of pX1108 and a partial Bam Island sequence of another X.laevis rDNA clone, pXlrl4 (22). It is clear that the Bam Island sequences from pX1108 and pXlrl4 are almost identical throughout their common homology.

Fig.5. A comparison of the Bam Island and 40S initiation site sequences. The sequences of Bam Islands 1 and 2 of pX1108, respectively lines 1 and 2, are shown aligned for best homology with the Bam Island sequence from pXlrl4 (22), line 3 and the 40S initiation site sequence of pX1108, line 4. Bases not falling into the homology are shown shaded.
with the 40S initiation site. However they differ significantly from each other near their boundaries with and just within the adjacent repetitive regions (Fig.5).

As previously demonstrated (7) rep.reg.2 of pX1108 consists of homologous 60 and 81 bp units. However, considering the homology between these units and the phasing imposed on them by the Bam Island homologies, the region is more simply seen as being composed of non-homologous 60 bp and 21 bp units. The region then contains exactly twelve 60 bp units and seven 21 bp units. From Fig.4 it can be seen that sequence variabilities only occur in the 60 bp units and then only at two positions in each unit (e.g. compare pos. 2623/2764 and 2596/2737). This clearly demonstrates how little the units have evolved from each other since "saltation" of the progenitor sequence occurred. The variabilities which are seen have obviously been spread throughout the 60 bp units fairly rapidly. The situation is most simply explained by postulating that the two observed point mutations occurred independently and were spread throughout the region by unequal cross-over during recombination (23). This explanation is supported by the observation that at least one of these sequence variabilities also occurs in an analogous repetitive region of pX1r14 (comparison of Fig.4 with (22)). However, two further observations argue against this as the sole explanation of spacer evolution. Firstly Boseley et al. (7) noted that rep.reg.3 of pX1108 differed from region 2 in that every 21 bp unit (see above) contained a Sma I restriction site not present in the rep.reg.2. The sequence data for pX1r14 (Fig.5) (22) shows what is probably this same "Sma I" mutation, but restriction data (21) for this clone and another independent clone pX1r15 indicate that here the mutation is probably confined to the Bam Island adjacent unit of each repetitive region. Thus in the case of pX1108 a "Sma I" mutation is confined to all units of one repetitive region while in the case of pX1r14 and 15 it is probably confined to only one unit of all analogous repetitive regions. This is clearly not to be expected if unequal cross-over operates throughout all spacer regions. Secondly, restriction data for two independent X.laevis rDNA clones (21) indicates that the length of a "Bam super-repeat" (i.e. Bam Island and repetitive region) is essentially constant within a given spacer but differs between spacers. This is also inconsistent with a simple model of unequal cross-over. Some aspects of the Keyl model of "reduplication and insertion" (24) would however be consistent with the above observations.
The repetitive region 3 - 40S initiation site boundary. Moving on downstream through the spacer, repetitive region 3 is separated from the Bam Island homologous 40S initiation region by a sequence of 116 bp (pos.4176-4291) (Fig.4) having no clear homology to sequences found elsewhere in the spacer. However, this 116 bp segment contains an oligo(dT) tract and following short sequence, homologous to the sequence immediately preceding the 40S initiation site (compare in Fig.4 pos.4209-4226 with 4410-4426). It is therefore possible that this 116 bp sequence is a remnant of an earlier initiation site duplication.

The external transcribed spacer and 18S gene. We have compared the available sequence data of S.cerevisiae rDNA (25,26) with that of X.laevis. The sequence data for S.cerevisiae includes most of the external transcribed spacer (ETS) and the 5' end of the 18S gene, which has been accurately mapped (26). From estimates of the length of the ETS in S.cerevisiae (27) it seems probable, however, that the 5' coding sequence for the 37S pre-rRNA may not be contained within these sequences. A comparison of the ETS sequences of X.laevis and S. cerevisiae revealed no significant homologies, as might be anticipated from their highly diverse AT:GC ratios. However the sequences near the 5' ends of the 18S genes show about 80% sequence homology (Fig.6). On the basis of this homology we have estimated by extrapolation the hypothetical position of the 5' terminus of the X.laevis gene. Its sequence has however not as yet been determined.

The striking homologies between the 18S coding sequences of S.cerevisiae and X.laevis show a strong evolutionary conservation which greatly contrasts with a lack of conservation of the ETS regions. It has previously been demonstrated that the ETS varies considerably in length between species (28, 29,30) and since, as we show, it may also vary greatly in primary structure it would appear that a major portion of the ETS may not perform an exacting

**Fig. 6.** Comparison of the sequences of X.laevis and S.cerevisiae (26) rDNAs in the regions of their 18S rRNA 5'-terminal coding sequences.
function. Bearing this in mind it is tempting to speculate on the possible evolution of the ETS.

It was argued earlier (7,8) that the NTS of X.laevis rDNA probably evolved in part by the reduplication of the presumptive promoter sequences and this appears also to be the case in Xenopus borealis (9). It has further been demonstrated that in E.coli the rRNA operons have duplicate promoters (31-33). Thus it might appear, that a general property of the ribosomal operon is to reduplicate its promoter sequence. If, however, after duplication of the promoter region the original promoter were to become inactive an ETS would essentially have been produced. It is then possible that the ETS observed in various species occur in part as a balance between deletion of a non-functional sequence and the reduplication of the functional promoter.

The DNA sequence coding for the 4OS 3' terminus. It has been demonstrated by electron microscope mapping experiments that the 3' terminal coding sequence of the X.laevis 40S pre-rRNA lies 500±50bp downstream from the EcoRI site of the 28S gene (34). These data place the terminal coding sequence between position 450 and 550 (Fig.4) near to the single HindIII site (pos. 502) of the rDNA repeat unit.

Analysis of the sequence surrounding the HindIII site shows that a sharp transition in the AT:GC ratio from 48% to 83% GC, sequence positions 410-510 and 510-610 respectively, occurs in its immediate vicinity. Since the X.laevis NTS has on average a GC content of about 80%, such a transition is itself consistent with the transition from gene to spacer.

The termination of transcription in E.coli-related rho-independent systems occurs almost invariably downstream of a GC rich sequence which when transcribed is usually capable of forming a stable hairpin loop (35,36). Recent data suggests that this could also hold for eukaryotic genes transcribed by polymerase II (18). Further E.coli-related rho-independent termination occurs within an oligo(dT) tract or AT rich sequence of six or more nucleotides (35,36) and a much longer poly(dT) tract may be involved in S. cerevisiae 5S rRNA termination (37).

Fig.7 shows the palindromic sequences, oligo(dT) tract and AT rich sequences lying in the region of the X.laevis 40S rRNA 3' terminal coding sequence. Recently the 40S 3' terminal coding sequence, essentially identical to that given in Fig.4 and 7, was determined for the X.laevis rDNA clone pXlr14 and the 40S rRNA 3' terminus mapped on this clone to position 503 in
Fig. 7. The palindromic sequences occurring in the 40S pre-rRNA 3' terminal coding region. Palindromes are indicated by the arrows and their stabilities as RNA hairpin structures (40) are given for those which are probably transcribed. AT-rich and T-rich segments are shown underlined.

Fig. 7 (22), i.e. immediately upstream of the oligo(dT)$_4$ tract. Inspection of Fig. 7 shows that although a palindromic sequence does occur immediately upstream of the termination sequences it is by no means especially GC rich and as RNA would not form a stable hairpin loop. Furthermore another oligo (dT)$_4$ tract occurs 425 bp upstream of the 40S termination site (T. Moss, unpublished) and is therefore alone insufficient to determine termination.

Interestingly it has been shown that in vitro termination of Tetrahymena rRNA synthesis is dependent on a low molecular weight protein factor (38), thus conceivably this could also be the case for X. laevis rRNA synthesis.

In conclusion, we have approached the problem of ribosomal gene spacer function by firstly analysing the primary structure of such a spacer from Xenopus laevis. This primary structure analysis has been instrumental in the identification of a putative promoter for the 4OS pre-rRNA transcript (7-9), has shown that this putative promoter is reduplicated (7,8) and has revealed the rather complex arrangement of repetitive and non-repetitive sequence elements within the spacer (7 and the present work).

The present work essentially completes the primary structure analysis of this ribosomal spacer and lays the foundation upon which detailed functional tests may be devised.

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