Xenopus laevis c-myc I and II genes: molecular structure and developmental expression

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ABSTRACT

The structure of the two *Xenopus laevis* c-myc I and c-myc II genes has been investigated by isolating and sequencing genomic and cDNAs clones. In oocytes, c-myc I mRNAs represent 80 – 90% of the overall amount of c-myc transcripts. The c-myc I expression is controlled primarily by two differentially regulated tandem promoters P1 and P2 which are separated by 50 bases. During oogenesis, maternal c-myc I mRNAs, are transcribed from both promoters whereas zygotic transcripts seem to initiate only from the P2 promoter. Sequence comparison between the promoter regions of c-myc I and II genes reveals the insertion in the c-myc I promoter region, between positions – 831 and – 389 relative to the P1 start site of a repetitive element. Comparison of *X.laevis* and mammalian c-myc promoter sequences reveals furthermore the conservation of cis-regulatory elements, including a motif known to be a negative regulator of the human c-myc transcription, a purine rich region, a binding site for the E2-F transcription factor and three SP1 binding sites. Finally, we report characterization of a new c-myc I mRNA which differ at the 5′ end. Transcripts are possibly initiated at a putative alternative promoter located further upstream in the genome, and undergoes alternative splicing.

INTRODUCTION

Recent analysis of early development of the frog *Xenopus laevis* has emphasized that growth factors, or related molecules, may act as morphogenic and differentiation signals (1). Interaction of growth factors with membrane receptors results in a cascade of biochemical changes that relay the signal to the cell nucleus and result in the expression of successive sets of genes.

The cellular homologue of the viral myc (v-myc) oncogene of the avian retrovirus MC29 is one of such growth-regulated gene which has been isolated from several vertebrates, including the frog *Xenopus laevis* (2,3,4). In proliferating and differentiating mammalian cells the c-myc gene is regulated both at transcriptional and post-transcriptional levels (5,6) and a number of protein factors interacting with the c-myc gene have been described (7,8,9,10,11,12,13). In *Xenopus* oocytes, the transcriptional activity of microinjected human c-myc gene constructs has been suggested to be dictated essentially by the interaction with its proximal promoter region of the SP1 protein (14). Chain elongation blockage at the 3′ end of the first exon has also be demonstrated for transcripts coded by microinjected human c-myc gene constructs (15,16).

X.laevis has undergone a genome duplication during its evolution (17). As a consequence, most genes are present in two copies. This has been shown also for the c-myc gene (2,3,4). Both c-myc genes are transcriptionally active during oocyte growth (18). *Xenopus* c-myc I mRNA is synthetized and accumulates massively during early oogenesis, and post-transcriptional regulation induced at fertilization is correlated with a degradation of over 90% of maternal c-myc mRNA by gastrula stage (4). It is also expressed in the growing embryo, and high mRNA levels are detected in specific tissues such as the epidermis and the lens (19). In contrast, c-myc II transcripts have not yet been described in post-gastrula embryos (18).

While there exist several studies (2,3,4 – 18,19) on the expression of the two *Xenopus* c-myc genes, isolation of the gene promoters has not been reported to date. Furthermore much remains to be understood of the molecular mechanisms underlying the c-myc I and II gene expression and the accumulation of maternal or zygotic transcripts. For example, it is not known whether *Xenopus* c-myc I and II genes are regulated during oogenesis concomittantly with the exogenous microinjected human c-myc gene, or whether the expression of the zygotic myc I and myc II transcripts are regulated in a manner similar to the maternal transcripts during the early development. In order to answer these questions, we cloned and analysed the promoter regions of the *Xenopus* c-myc genes together with the previously uncharacterized exons 1 and introns 1.

While both *Xenopus* c-myc genes are expressed in oocytes, embryos and adult tissues, the maternal c-myc transcripts are preferentially transcribed from the c-myc I gene, and these continue to predominate those from c-myc II in growing embryos and adult tissues. We demonstrate that maternal c-myc I transcripts are initiated from two main promoters named P1 and P2. In embryos and in adult tissues the pattern of expression of these genes involves only the P2 start site. Maternal and zygotic c-myc II transcripts initiating at the P2 promoter have also been detected. Furthermore, the results obtained after microinjection of c-myc II transcripts into oocytes suggest that the c-myc II P1 promoter might also be active during oogenesis. *Xenopus* c-myc I cDNA sequences also contain another set of molecules with the same exons 2 and 3, but a different sequence at the

5'untranslated end. This suggests that there may exist yet another promoter located further upstream in the genome thereby favouring the hypothesis of an alternative splicing.

MATERIALS AND METHODS

Oocytes, eggs and embryos

Xenopus laevis oocytes, eggs and embryos were collected and staged as described (20,21,22).

Molecular cloning and sequencing

A *X.laevis* egg cDNA library in lambda gt10 and a genomic library prepared in our laboratory (23) were screened with a probe prepared from a fragment of the cDNA clone pXlmycA (2). Hybridizations were performed in 5×SSC, 42% deionized formamide 1×Denhardt, 50 mM Phophate buffer pH 6,5 and 100 μg/ml sperm DNA at 37°C. Filters were washed twice in 0,1×SSC, 0.1×SDS at 42°C. Recombinant DNAs from positive phages were isolated according to (24). The Maxam and Gilbert technique (25) was used for sequencing both strands.

RNA 5'end mapping

RNAs were obtained by either the guanidium hydrochloride method or by lysis in proteinase K/SDS solution, followed by phenol extraction and precipitation with 2.5 M LiCl (24). RNA probes were synthesized using the T3 RNA polymerase and the method of Melton et al. (26). pVZXmycI contains a 350 bp Sac I/Sma I fragment (positions −138, +212 in Fig. 2) cloned into pVZ1 (27). pVZXmycII contains a 256 bp Sac I/Hinf I fragment (positions −136, +120). The relative amount of total RNA in each sample was assessed using a *X.laevis* 5S probe (28). RNAase protection analysis were performed as described (29). The probes were hybridized to 20 μg of total RNA at 42 or 50°C for 12 h and then digested with RNases A and T1 for 1 h at 30°C.

In ovo promoter assay

Bam H1 linkers were added to the ends of a 1355 bp c-myc I Pvu II fragment (−1258, +97 in Fig. 1) and of a 1168 bp c-myc II Eco RI/Hinf I fragment (−1048, +120). The resulting fragments were then cloned into the Bam H1 site upstream from the chloramphenicolacetyltransferase (CAT) gene of the plasmid pBLCAT3T. pBLCAT3T was constructed in order to minimize readthrough transcription from cryptic promoters (30,31). It was obtained as follow; a 300 bp Sal I/Hind III fragment was purified from the plasmid pU2/247/RA-2. This fragment contains consensus termination sequences for both RNA polymerases II and III (32). Pst I linkers were added to the blunt-ended ends of the fragment which was then cloned into the Pst I site of pBLCAT3 (33).

Oocytes were microinjected (34) and CAT activity was assayed in extracts of 5 oocytes (35).

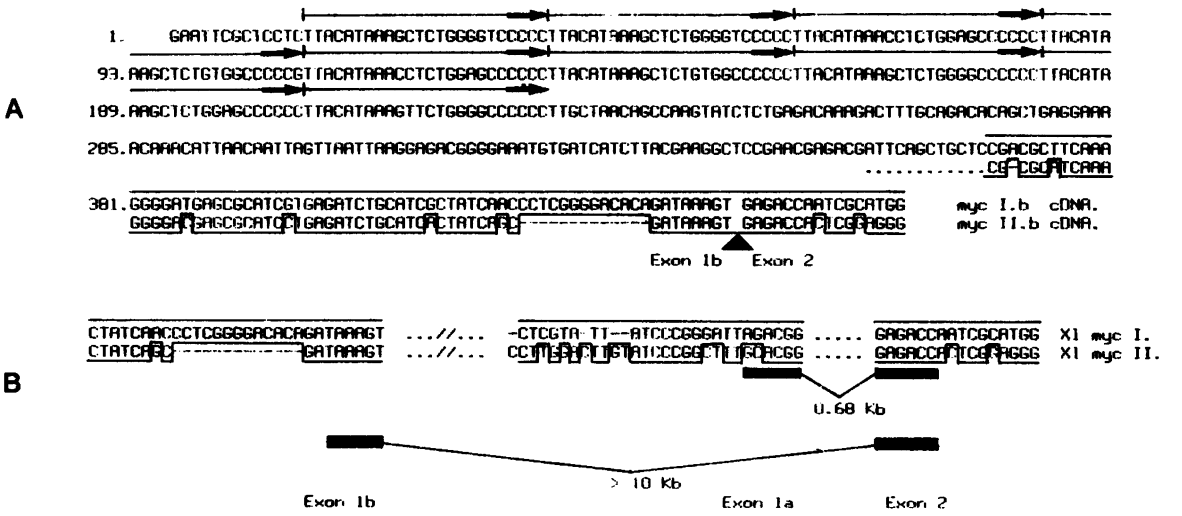
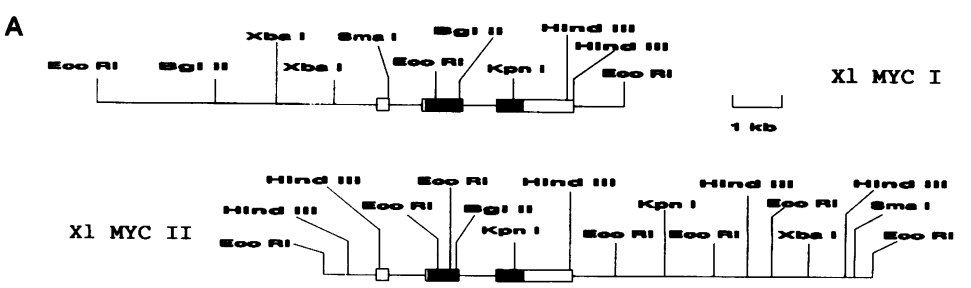


Figure 1. Nucleotide sequence of *Xenopus laevis* c-myc Ib and c-myc IIb cDNAs and schematic organization of the two c-myc genes. **Panel A:** Complete nucleotide sequence (433 bp) of our *Xenopus* c-myc Ib cDNA and comparison with the 5' end of the cDNA sequence, c-myc IIb (18). Arrows indicate repeats in the c-myc Ib cDNA sequence. Solid triangle indicates the exon1/exon2 boundary. **Panel B:** Organization of the Xl myc I and II genes. Short sequences corresponding to the 3' ends of exons 1a and 1b and to the 5' part of exon 2 are shown and compared between the two genes. Solid boxes below the sequences correspond to the limits of the different exons. Introns shown as bent lines are not drawn to scale.



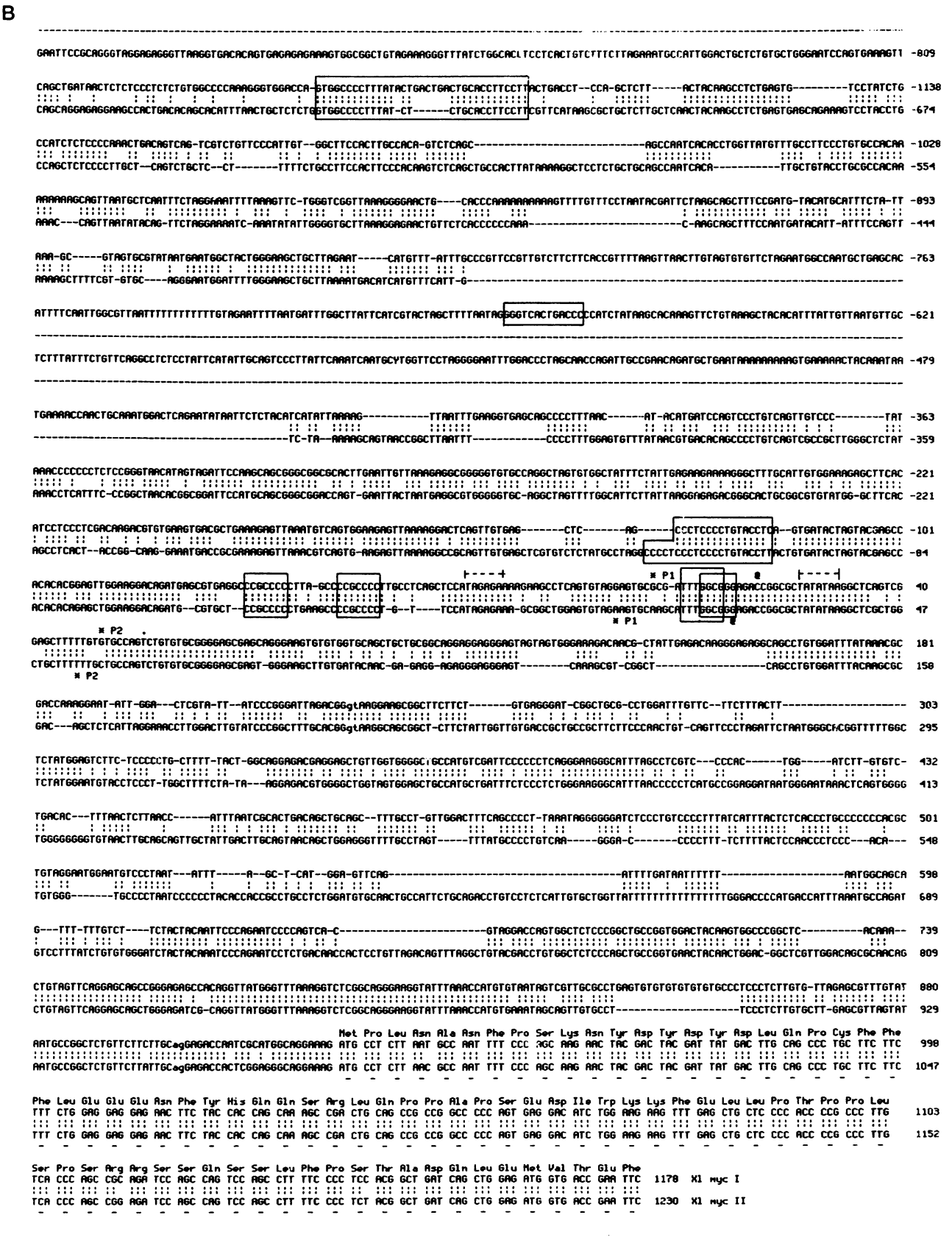


Figure 2. Restriction map and sequence alignment of *Xenopus laevis* c-myc I and II genes. **Panel A:** Restriction maps of lambda Xl myc I and II. The boxes represent exons and the solid portions denote the translational reading frame. **Panel B:** Sequence alignment of Xl myc I and Xl myc II. Gaps (--) have been introduced to permit optimal alignment. Two main start sites (*) are shown for both genes. Nucleotides are numbered with respect to assigned P1 transcription start sites. @ indicate the 5′ ends of our corresponding c-myc Ia and c-myc IIa cDNAs. Intron/exon boundaries are shown in lower case. Conserved regions between *Xenopus* and mammalian promoters are shown as boxes. Amino acids present at the N-terminus of both *Xenopus* MYC protein are also shown.

For primer extension assays, total RNA from 10 microinjected oocytes was hybridized to a CAT specific oligonucleotide primer 5'-TTTTCAGGAGCTAAGGAAGC-3' complementary to the CAT sequence. Primer labelling with polynucleotide kinase, hybridization, elongation and subsequent purification were as described (24).

RESULTS

Isolation and characterization of *X.laevis* c-myc transcription units

A *Xenopus laevis* cDNA library was screened for c-myc coding sequences (see methods) and nine cDNA clones were isolated. Among these, five are homologous to the c-myc I mRNA species previously described (2,3,4,18) and were designated as c-myc Ia (data not shown).

Three other clones, designated as c-myc IIa, correspond to a distinct species of c-myc mRNA with a near complete homology to the coding frame in c-myc II mRNA (18). The largest of our c-myc IIa cDNA clone (EMBL accession number×56870) is 2487 bp long, contains a single open reading frame coding for a protein of 421 amino acids and begins 213 nucleotides further 5' of the translation initiation codon. Alignment of the previously published *Xenopus* c-myc I cDNA (2−4-18) with our c-myc IIa cDNA shows 94% homology in the coding regions. The non-coding exon 1 of c-myc IIa itself presents 77% homology with the corresponding region of the c-myc Ia cDNA (see Figure 2b).

In our ninth cDNA clone, c-myc Ib, nucleotide sequences of exons 2 and 3, but not exon 1, are identical to the corresponding regions of c-myc Ia cDNA (Fig. 1). On the other hand, the exon 1 sequence shows (Fig. 1) considerable homology with the 5' region (nucleotides 1−63) of the XLMYCII clone (18) which has been isolated from a different cDNA library. The nucleotide sequence of exon 1 from clone c-myc Ib is 439 bp long and is characterized by the presence at its 5'end of nine consecutive repeats of 24 nucleotides (positions 14−229), which are not found in any of the previously known nucleotide sequences in the EMBL gene data bank. The exon 1 does not contain initiation codons. However, two open reading frames exist, followed by translation terminator codons 3' of the tandem repeats.

Cloning and sequencing of the c-myc I and II promoter regions

We have isolated genomic clones c-myc I and II designated as Xl myc I and Xl myc II, respectively, (Fig. 2a) and sequenced (Fig. 2b) 1.2 kb upstream from the first assigned transcription start site, the first exon, the first intron and part of the second exon. These genomic DNA sequences Xl myc I and Xl myc II include c-myc Ia and c-myc IIa cDNA sequences, repectively. We could not find the alternative exon 1 (Fig. 1a) present in the c-myc Ib cDNA clone neither within the genomic clone Xl myc I nor in another genomic clone extending approximately 7 kbp more upstream.

Localization of the 5' ends of c-myc I and II transcription units

RNAase mapping experiments were carried out to locate the transcription initiation sites. Several such sites were identified

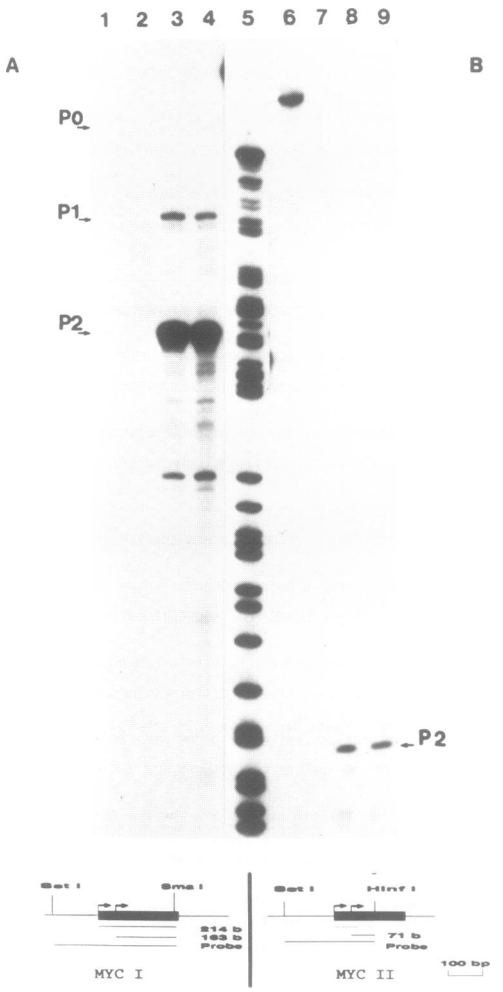


Figure 3. RNase protection analysis. Antisense RNA from either the 350-bp Sst I/Sma I Xl myc I restriction fragment (panel A) or the 256 bp Sst I/Hinf I Xl myc II restriction fragment (panel B) were hybridized with total oocyte RNA either at 42°C (panel A, lane 3; panel B, lane 8) or at 50°C (panel A, lane 4; panel B, lane 9). As negative control, tRNA was used (lanes 1−2-6−7).

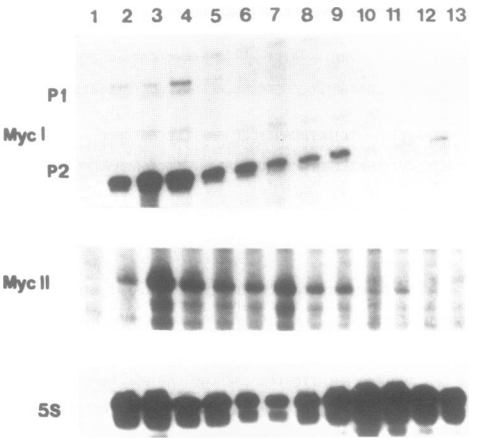


Figure 4. RNase protection assay to monitor the level of c-myc I and II transcripts during development. RNA samples (20 μg) were hybridized at 42°C with antisense RNA probes for Xl myc I (upper panel), Xl myc II (middle panel) and 5S RNAs (lower panel). The positions of transcripts initiating from the two promoters in Xl myc I are shown. The middle and lower panels show the major protected fragments obtained with the Xl myc II and 5S RNA probes. Lane 1, tRNA, lanes 2−5, oocyte RNA (total, stages 1−2, stages 3,4, stages 5,6 respectively), lane 6, unfertilized egg RNA, lanes 7−9, gastrula, neurula and tadpole RNA respectively, lanes 10−13, liver, skin, heart and muscle RNA respectively.

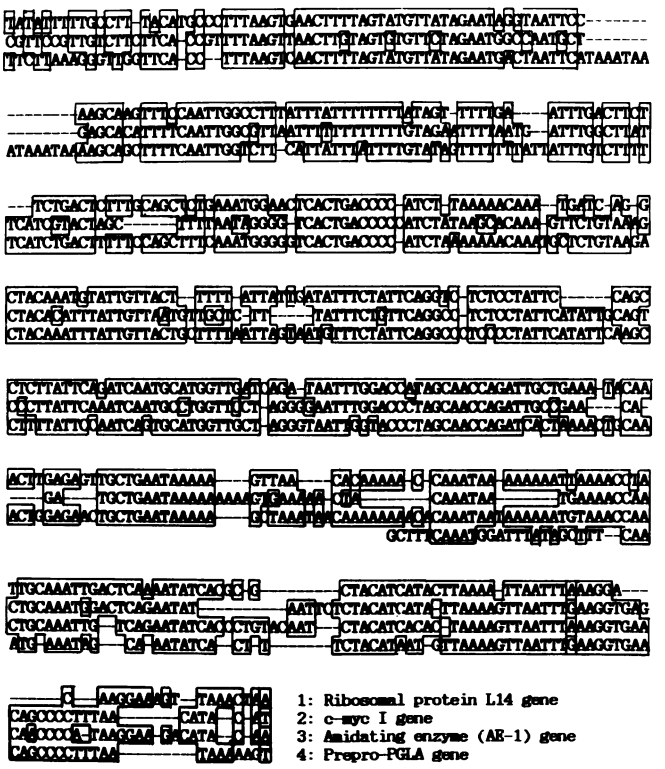


Figure 5. Nucleotide sequence comparison of a region of the *Xenopus* Xl myc I promoter region (−389, −831 relative to the P1 transcription initiation site) with three other *Xenopus laevis* sequences. *Xenopus* sequences compared are 1) part of intron 5 (6189,6622) of ribosomal protein L14 gene (50); 2) 3′ end (1930,2434) of α-amidating enzyme gene (51); 3) part of intron 1 (31,131) of prepro-PGLA skin peptide gene (52). Gaps (−) have been introduced to maximise the matches among sequences.

(Fig. 3) after hybridization of oocyte RNA with complementary RNA transcribed in vitro from the Xl myc I DNA (see methods). Two major start sites P1 and P2, are located 226 and 175 bases upstream, respectively, from the exon 1/intron 1 boundary. This agrees with the localization of the 5′ end of c-myc I transcription unit obtained by primer extension experiments (18). The sequences equivalent to TATA boxes are located between −26 and −20 or −24 and −18 bases upstream respectively. We also find (Fig. 3) at least two weak signals suggestive of minor start sites (P0) further upstream.

Similar experiments were also performed using a Xl myc II RNA probe (see methods). The 5′ most major protected fragment, located 153 bases upstream from the exon 1/intron 1 boundary correspond to an mRNA species initiating at the promoter P2 (Fig. 3).

Expression of c-myc I and II during Xenopus early development

We used RNase protection assay to detect c-myc I and II transcripts during the development of *Xenopus*. Maternal and zygotic transcripts initiating at the P2 promoter of Xl myc I were seen during oogenesis, embryonic development and in adult tissues (Fig. 4A). Transcripts presumably initiating from the more upstream promoters (P1 and P0) were observed only during oocyte growth. The relative intensity of hybridized RNA was maximal for the earliest oocytes and decreased with oocyte development. From gastrula stage on, c-myc I RNA is maintained constitutively. These transcripts, initiated at the P2 start site, have also been observed in liver, skin, heart and muscle.

A similar transcript distribution was observed when total RNA from oocytes, embryos or adult tissues were hybridized with a c-myc II-specific probe (Fig. 4B). However, the level of c-myc II transcripts initiating at the P2 promoter was approximately

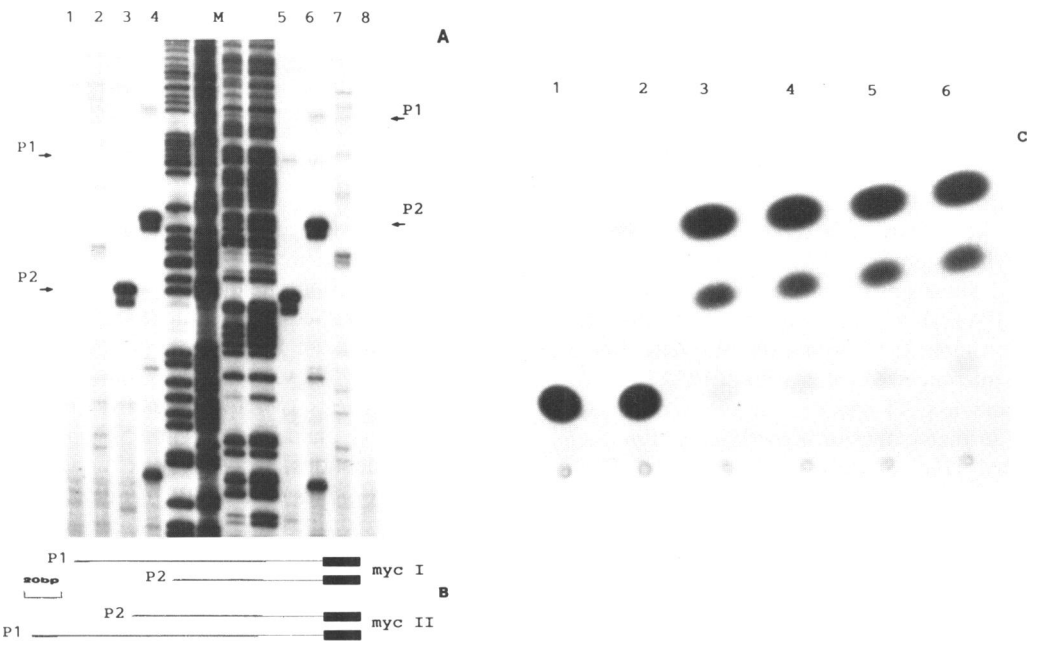


Figure 6. Promoter activity of the two *Xenopus* myc genes. **Panel A:** Primer extension experiments. pBLCAT3T vectors containing promoter sequences of *Xenopus* Xl myc I (lanes 3,5) or Xl myc II (lanes 4,6) (see methods) were microinjected in oocytes in two separate experiments. myc-CAT fusion transcripts were detected with a CAT specific oligonucleotide. Experiments with negative control oocytes (lanes 1,8) or those injected with the vector pBLCAT3T alone (lanes 2,7) are also shown. **Panel B:** Summary of the primer extension analysis. **Panel C:** CAT activity of the Xl mycCAT fused genes. Oocytes were injected with pBLCAT3T (1,2) or pBLCAT3T vectors containing *Xenopus* Xl myc I (3,4) or Xl myc II (5,6) sequences and CAT activity examined on TLC plates (see methods).

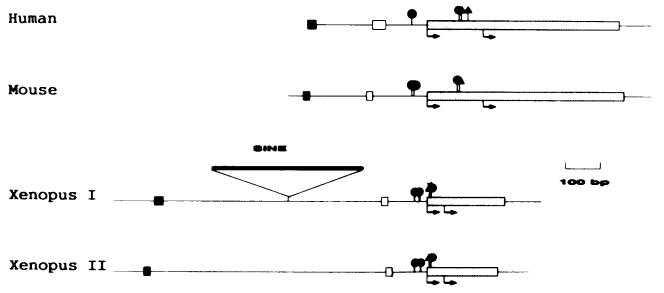


Figure 7. Comparison of vertebrate c-myc promoter sequences. The diagram illustrates the topography of human, mouse and *Xenopus laevis* myc promoters drawn to scale. Exon 1 is shown as stippled box. Arrows indicate the sites for transcription initiation controlled by promoters P1 and P2. Locations of regulatory elements controlling the expression of mammalian c-myc genes are derived from the literature (8,9,10,12,13). Solid boxes show location of the negative regulatory element for human c-myc and related sequences in mouse and *Xenopus* genes. Open boxes denote pyrimidine-rich sequences. (●) E2F binding sites. (▲) SP1 binding sites. The insertion of a 500 base repetitive sequence (SINE) is also shown above the *Xenopus* Xl myc I sequence.

10−20% of that of c-myc I RNA . Maternal transcripts initiating at the P1 promoter were practically undetectable in these experiments (see discussion).

Insertional mutagenesis of the c-myc I locus by a repetitive, SINE-related sequence

Sequence comparison (Fig. 2b) between the two *Xenopus laevis* myc genes revealed a 500 bp region (−831, −389 relative to the P1 initiation site) present only in Xl myc I. A search of EMBL gene sequence data bank revealed a significant match (over 65%) between this 500 bp segment and three other *Xenopus laevis* sequences located in introns or at the 3′ end of genes (Figure 5). In all four cases, this region exhibits features characteristic of the SINE family of transposable elements (36) containing short (500 bp or less) interspersed elements, without open reading frames and, with 3′ ends rich in A-residues, containing one or more polyadenylation signals.

Promoter activity

To determine c-myc I and II promoter activity, the regions of the Xl myc I and II from −1258 to +212, and from −1048 to +120 respectively were fused to a reporter gene encoding chloramphenicol acetyltransferase (CAT) and microinjected in oocytes (see methods). These upstream sequences promoted an efficient synthesis of CAT (Fig. 6C), while the control vector pBLCAT3T did not promote CAT synthesis. We used RNAs synthesized in oocytes microinjected either with pBLCAT3TmycI or pBLCAT3mycII and a CAT-specific oligonucleotide (see methods) to perform primer extension experiments. For each construct, we detected (Fig. 6A, 6B) two extended DNA fragments, whose sizes indicate that transcription starts at the P1 and P2 promoters. Control assays, with tRNA or RNA from oocytes microinjected with pBLCAT3T, did not sustain the synthesis of such elongation products (Fig. 6A).

DISCUSSION

We have isolated the genomic clones Xl myc I and Xl myc II containing promoter elements (Fig. 2) and nucleotide sequences corresponding to previously described c-myc cDNAs (2,3,4,18). The c-myc I and II polypeptide encoding sequences are highly

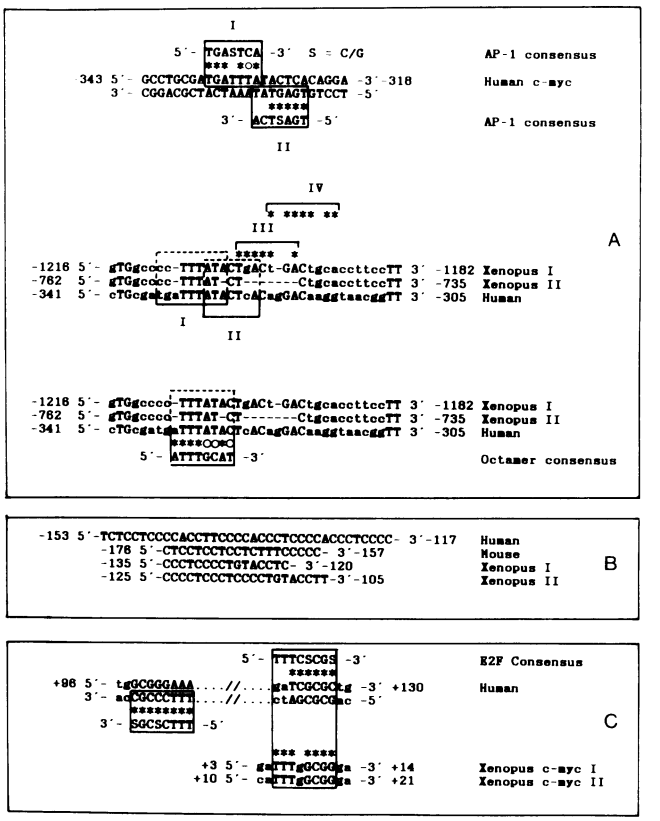


Table I. Homology of *Xenopus laevis* Xl myc I and Xl myc II to the nuclear factor binding sites of human c-myc. Nucleotide positions are defined relative to the site of initiation for P1 promoters. (*) an identically matched base, (○) a purine or pyrimidine match. **Panel A:** Similarities between human c-myc negative response element (NRE) and *Xenopus* c-myc promoter sequences. In the uppermost panel consensus AP-1 binding sites (I and II) were aligned with the human 26-bp negative element (12). Middle panel: shows alignment of the human NRE including AP-1 binding sites and homologous *Xenopus* Xl myc I and II containing AP-1 like binding sites (III and IV). Lower panel: A consensus octamer binding site (12) is aligned with human NRE and homologous *Xenopus* Xl myc I and Xl myc II sequences. **Panel B:** Nucleotide sequence alignment of mammalian and *Xenopus* myc sequences showing the pyrimidine-rich region **Panel C:** Comparison of the E2F binding sites in the human myc gene (13) with related sequences in the *Xenopus* Xl myc I and II promoters.

conserved. Conserved regions are also evident in the promoter and 5′ non-coding areas. Exons I of *Xenopus* c-myc I and II are significantly shorter than the mammalian homologs and have no coding capacity. Sequence comparison between mammalian or chicken c-myc exon 1 and the same region in *Xenopus* fails to reveal any homology.

During oogenesis, two *Xenopus* c-myc I transcripts initiate from the promoters P1 and P2 and bear a 5′ non coding region of 251 and 199 nucleotides, respectively (Fig. 3). As in mouse and human c-myc genes (37,38,39), c-myc I transcripts initiating (Fig. 3) approximately 100 bp upstream from P1 have also be detected. Initiation from the P1 and P0 promoters seem to be a distinctive feature of maternal transcripts since we failed (Fig. 4) to detect these in embryos even after longer exposures.

The c-myc II gene is expressed (Fig. 3) during oogenesis from a promoter P2 located 177 bp upstream from the ATG. We think that usage during oogenesis of a P1 promoter located 228 bp upstream from the ATG is also likely since we have isolated, from an egg cDNA library, a cDNA clone (EMBL accession number: X56870) representative of an mRNA initiating upstream

from the P2 start site and we have detected P1-initiating myc II-CAT transcripts after microinjection of such fusion reporter genes into oocytes (Fig. 6A).

We have also detected c-myc II transcripts initiating from the P2 promoter in embryos and in adult tissues (Fig. 4). These data contrast with earlier results (18) in which, a 2.7 kbp long c-myc II mRNA initiating from a 'P'' promoter and containing a 410 bp 5' untranslated region was detected only during oogenesis.

The exon 1 sequence of our genomic clone Xl myc I corresponds to two c-myc I cDNA sequences previously described (2,18). These two cDNAs start 66 and 117 bp downstream from the P2 transcription initiation site respectively. The exon 1 nucleotide sequence of these cDNAs is almost identical to the sequence of our genomic clone, except for the very 5' end of one of them (2) which presents a difference of five nucleotides. This is likely to be due to the cloning procedure. We have not determined the nucleotide sequence of the 3' part of our genomic clone. This could be helpful to find out the reason of the various nucleotide sequence discrepancies present in the 3' untranslated region of the two published cDNAs (2,18).

We have compared the sequence of our c-myc IIa cDNA clone with the previously published sequence XLMYCII (18) and found seven differences in the second and third exons between the c-myc II.a sequence and the previously published c-myc II sequence (18). Among these, three (positions 754, 988, 1093 in our c-myc IIa sequence) do not affect the coding properties while, two other, namely replacement of nucleotides 772 and 1188, results in codon modification. The last case, concerns the deletion of a G at position 1310 and the insertion of a T at position 1319. This results in a frameshift extending over three amino-acids, so that codons for Glu/Val/Ala present in our cDNA have been replaced by Arg/Trp/Arg. It is interesting to note that in our hands the region between 1310 and 1319 for c-myc Ia and c-myc IIa are completely homologous and code for Glu/Val/Ala.

The exon 1 of c-myc IIa is 228 bp long and the sequence has no homology with the 63 bp exon 1 sequence previously described (18) for c-myc II cDNA. We feel that the location of this divergence might be either accidental, or that a splicing event might have arisen in which the normal noncoding exon 1 was replaced by another exon lying 5' to it in the genome. We favour this last explanation since we do find (Fig. 1A) at the 3' end of the c-myc Ib exon 1, a 71 bp sequence homologous (70%) to the first 63 bp of XLMYCII (18). Therefore, the two cDNA clones could correspond to alternatively spliced mRNAs transcribed from the c-myc I and c-myc II genes, respectively. This hypothesis needs however additional confirmation, but it is interesting to point out that a c-myc cDNA corresponding to an alternatively spliced transcript has been reported also in human B-cell lymphomas (37). The 5' end of our c-myc Ib clone containing the above exon 1 is highly structured with an unusual ninefold repetition of a 24 bp motif for which we could not find a homologous sequence in the EMBL bank. The significance of this repeated sequence motif remains unknown.

Similar to mouse and human c-myc genes in which transcripts initiating upstream from the 5' promoter (P1) can be detected, we have also observed such weak P0 start sites in *Xenopus*. However, we do not find any sequence corresponding to exon 1 of c-myc Ib in the promoter areas of Xl myc I and Xl myc II or in the case of Xl myc I up to 10 kbp upstream. Therefore, we think that the structure of the c-myc gene in *Xenopus* is more complex than that described in mammals.

Alignment of 5' sequences of our genomic clones Xl myc I and Xl myc II reveals the presence in the former of a 500 bp repetitive DNA element. Sequences homologous to this DNA are present in three other *Xenopus* genes (Fig. 5). Such short elements with variable 5' ends and common A-rich 3'ends are reminiscent of human SINE Alu sequences (36) and form a new family of repetitive elements which have not been reported (40) so far in amphibians. We also observe (Fig. 4) that the amount of c-myc I transcripts is significantly greater than that for c-myc II during oogenesis and development and it will be interesting to know whether this is due to the presence of the repetitive element in the Xl myc I gene. It is interesting to note that a perfect consensus sequence for the thyroid hormone and retinoic acid responsive elements (5'-GGTCAgTGACC-3') (41) is also found in this repetitive sequence. This makes us wonder whether the insertion of this sequence into the control region of a developmentally regulated gene might modify its expression. Since thyroid hormone and retinoids have been implicated in the metamorphosis and antero-posterior patterning of the embryo, respectively (42), it would be interesting to study the expression of the c-myc I gene during these processes.

A comparison of nucleotide sequence of human and mouse c-myc promoters *Xenopus* c-myc I and c-myc II shows (Fig. 7; Table I) that some of the cis-regulatory sequences are conserved. Cis-regulatory sequences most proximal to the TATA boxes are SP1 binding sites located immediately upstream of P1 and P2 which have been shown (14) to be essential for basal human c-myc promoter activity in *Xenopus* oocytes.

In human and mouse c-myc genes, binding sites for the E2F cellular transcription factor have been found just upstream from the P2 TATA box. This factor interacting with two sites in the adenovirus E2 promoter is involved in the transactivation of human c-myc by adenovirus E1A proteins in transient assays (10,13). We find that (Table I, panel C) this site is conserved in the c-myc I and II genomic sequences. Further upstream between positions −135 to −120 in the c-myc I gene and −125 to −105 in the c-myc II gene lies a pyrimidine-rich element in the sense strand with direct repeats of tri and tetra-C/T dinucleotides (Table 1, panel B). Such an element has been described (43,44,45,46) in the promoter region of genes coding for the epidermal growth factor receptor (44), the insulin receptor (46) and the oncoproteins Ki-Ras and Ets 2 (43,45) among others, and has been shown to enhance (7,8,47) the transcription from the human or mouse P2 promoters and to interact with protein factors, including a ribonucleoprotein (8).

A 29 bp long negative regulatory element has been localized (9,12) approximately 300 bp upstream from the human c-myc promoter P1 which interacts with fos/jun (9,12) and an octamer-binding protein (12). We detect, (Table I, panel A) 1.2 kb upstream from the c-myc I promoter P2 and 0.75 kb upstream from the c-myc II promoter P2 a similar 30 bp segment containing a sequence possibly related to the sequence element found to interact with an octamer-binding protein in the human promoter (12) (Table I, panel A), while the AP-1 binding site is not found in the frog genes. On the other hand, as compared to the AP-1 binding consensus site (5'-TGAC/GTCA-3'), we do observe (Table I, panel A) the presence of a motif 5'-TGACTGACTG-CAC-3' which could represent a binding site for AP-1 related protein complexes. In this 30 nucleotides segment of *Xenopus* genes, we also find a motif (5'-CTTCCT-3') which is the same as the PEA3 motif of the polyoma enhancer involved in binding of the p68 c-ets-1 protein (48) which presumably cooperates with

the AP-1 complex for activation of transcription (49). A functional role for such sequences is actually studied in our laboratory, preliminary results indicates that the region located between the two promoters and containing overlapping SP1 and E2F binding sites is essential for the transcription of the c-myc I gene into oocytes.

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