Volume 232, number 1, 227–234 FEB 05863 May 1988

Organization of 5′-proximal *c-myb* exons in chicken DNA

Implications for *c-myb* tissue-specific transcription

Johann Soret, Michel Vellard, Cécile Martinerie and Bernard Perbal

Institut Curie, Bâtiment 110, Centre Universitaire, 91405 Orsay, France

Received 22 February 1988; revised version received 23 March 1988

The organization of 5′-proximal *c-myb* exons in chicken DNA has been established by restriction enzyme mapping and nucleotide sequencing. Hybridization studies performed with cDNA probes revealed that yolk sac and thymic *c-myb* RNAs differ in their 5′-termini. A comparison of the genomic *c-myb* sequence with that of cDNAs isolated from normal thymic and lymphoma avian cells suggests that different promoter regions are used to initiate *c-myb* transcription in hematopoietic cells of different origins.

c-myb exon; Avian genome; cDNA sequence; Nucleotide sequence; Lymphoma; Transcription; (Thymus, Yolk sac)

1. INTRODUCTION

The avian *c-myb* proto-oncogene is the cellular progenitor of the *v-myb* oncogene transduced by avian myeloblastosis virus and avian erythroblastosis virus E26. The viral sequences only represent a fraction of the cellular gene [1,2] whose precise boundaries in the genome have yet to be determined. Both chicken *c-myb* and viral *v-myb* products are nuclear proteins of 75 and 45 kDa, respectively [3–5]. The *c-myb* gene appears to have been conserved during evolution, as shown by the isolation and characterization of homologous genes in human, mouse and fruitfly DNA [6–8].

There is also a growing body of evidence suggesting that the *c-myb* product is involved in differentiation and proliferation of hematopoietic cells. For example, increased levels of *c-myb* transcripts have been detected following stimulation of mature human resting T cells [9–11] and transcription of *c-myb* has been reported primarily in immature hematopoietic cells of all lineages [12,13], at all phases of the cell cycle [14]. Consistent with these observations, elevated levels of *c-myb* RNA transcripts were detected in yolk sac cells and thymus [12,15] in accordance with the chronology of hematopoiesis in yolk sac, thymus, bone marrow, and spleen during the embryonic and early post-embryonic period in chicken [16].

To date, different *c-myb* cDNAs have been isolated from avian B cell lymphoma [17], avian normal thymic cells [15], murine pre-B cell lymphoma [18], murine myeloid leukemia [7] and human T lymphoma [19]. Interestingly, the proteins encoded by the lymphoma and myeloid leukemia cDNAs are very similar, while they differ significantly in their amino terminus from the protein encoded by the normal chicken thymic cells cDNA (fig.1).

As a first step in our study of the factors governing the expression of the *c-myb* proto-oncogene during differentiation, we have undertaken a characterization of the 5′-proximal *c-myb* exons and corresponding regulatory sequences in chicken DNA.

Correspondence address: B. Perbal, Institut Curie, Bâtiment 110, Centre Universitaire, 91405 Orsay, France

The nucleotide sequences presented here have been submitted to the EMBL/GenBank database under the accession no.Y00984

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

```
                                        Met Ala Arg Arg Pro Arg His Ser Ile Tyr Ser Ser Asp ... HUMAN
... ... AGC CCC GCC GCC CGC CGC GCC ATG GCC CGA AGA CCC CGG CAC AGC ATA TAT AGC AGT GAC ... (Lymphoma)

                                        Met Ala Arg Arg Pro Arg His Ser Ile Tyr Ser Ser Asp ... MURINE
... ... CGG AGC CCG GCC CGC CTC GCC ATG GCC CGG AGA CCC CGA CAC AGC ATC TAC AGT AGC GAT ... (Lymphoma)

                                        Met Ala Arg Arg Pro Arg His Ser Ile Tyr Ser Ser Asp ... MURINE
... ... GGA GCC CCG GCC CGC CTC GCC ATG GCC CGG AGA CCC CGA CAC AGC ATC TAC AGT AGC GAT ... (Leukemia)

                                        Met Ala Arg Arg Pro Arg His Ser Ile Tyr Ser Ser Asp ... AVIAN
... ... TCC CCC AGC GGC CGC CGG AGG ATG GCC CGG AGA CCC CGG CAC AGC ATA TAC AGC AGC GAT ... (Lymphoma)

Met Ala Ser Ile ... Pro Pro Ala Ala Ala Ala Arg Met Ala Arg Arg Pro Arg His Ser Ile Tyr Ser Ser Asp ... AVIAN
ATG GCG TCC ATC ... CCC CCA GCG GCC GCC GCG AGG ATG GCC CGG AGA CCC CGG CAC AGC ATA TAC AGC AGC GAT ... (Normal thymus)
```

Fig.1. Structural comparison of the amino-terminal portion of *c-myb* proteins encoded by tumoral and normal tissues from different origins. The putative proteins encoded by the RNA expressed in lymphoma and leukemia cells of different origins show a very high degree of homology. Only their amino termini have been represented, with part of the corresponding leader sequences. The normal avian thymic protein would contain 58 additional amino acids. The ATG initiation codon recognized in other proteins encodes an internal methionine in thymic *c-myb* RNA.

Here, we report the genomic organization and sequencing of 5'-proximal *c-myb* exons in chicken DNA with the localisation of the ATG initiation codon previously identified in the *c-myb* cDNA from avian B cell lymphoma. We also show that the thymic *c-myb* cDNA profoundly diverges in its 5'-terminus from the *c-myb* mRNA species expressed in normal yolk sac hematopoietic cells.

2. MATERIALS AND METHODS

2.1. *Preparation of polyadenylated RNA from normal hematopoietic chicken cells: agarose gels and transfer to nitrocellulose*

Yolk sac cells expressing the *c-myb* 4.0 kb mRNA species were prepared from 13-day-old embryos as described [20]. Total and polyadenylated RNAs were prepared according to standard methods [21]. RNA species were separated on 1% agarose gels containing formaldehyde [21] and transferred onto nitrocellulose for hybridization with specific probes [21].

2.2. *Labeling of probes and hybridization*

All probes were prepared from cloned fragments. The DNA inserts were purified following digestion with appropriate restriction endonucleases, electrophoresis in agarose or polyacrylamide gels (depending on the size of the fragment to be purified) and elution under the conditions in [21].

Labeling of DNA longer than 300 bp was performed by nick translation in the presence of [α-^{32}P]dCTP. Short DNA fragments were labeled with [α-^{32}P]dCTP by using the Boehringer random priming kit.

2.3. *Cloning of DNA fragments: digestion with restriction endonucleases*

Preparation of DNA fragments and selection of recombinant clones were performed as in [21]. The recipient vectors were pBR322, pUC18, M13 mp18 and M13 mp19.

Restriction endonucleases were purchased from New England Biolabs or Bethesda Research Laboratories and used under the conditions recommended by the suppliers.

2.4. *DNA sequencing*

Sequencing was performed by the dideoxy chain-termination method of Sanger et al. as described elsewhere [21]. Sequencing primers were purchased from New England Biolabs. (α-^{35}S)-labeled dATP (New England Nuclear) and the Klenow fragment of *E. coli* DNA polymerase I (Boehringer or New England Biolabs) were used in sequencing reactions. Sequence data treatments were performed using the computer facilities at CITI2 in Paris on a PDP8 computer, with the help of the French Ministère de la Recherche et de la Technologie (Programme mobilisateur 'Essor des Biotechnologies').

3. RESULTS

3.1. *Organization of the 5'-proximal* c-myb *exons in chicken DNA*

3.1.1. Identification of genomic DNA fragments containing *c-myb* sequences homologous to yolk sac RNA

DNA sequences homologous to the AMV *v-myb* oncogene were previously mapped on the chicken genome [1,2,22,23]. Several λ recombinant con-

taining genomic fragments located upstream and downstream of these *c-myb* sequences were characterized [2]. Two of them (λ1041, λ121) were shown to contain the *c-myb* exon corresponding to the 5'-proximal *v-myb* sequences of AMV [1] in a 628 bp *Pst*I fragment located at the 3'-end of the cellular insert (fig.2). λ1041 DNA was digested with several restriction endonucleases and a partial restriction map was established (fig.2). Five different fragments generated after a double *Pst*I-*Eco*RI digestion of P542 DNA [2] were subcloned in pUC18 (EP180, Pst049, Pst174, Pst063, PE080), radiolabeled by nick translation and used to probe *c-myb* RNA species in yolk sac RNA. The results obtained (not shown) revealed that in addition to those contained in Pst063, the *c-myb* sequences were contained in Pst174 and Pst049 DNA. A similar approach performed with P804 DNA did not permit the detection of any DNA sequences homologous to yolk sac RNA in this 8.0 kb fragment.

3.1.2. Sequencing of the 5'-proximal *c-myb* exons

The strategy followed for sequencing is depicted in fig.2. The identification of the different *c-myb* exons in P542 and P804 DNA could be established by comparing our sequence data with the cDNA sequences previously published. Three *c-myb* exons were mapped upstream to that located in Pst063 (and corresponding to the 5'-end of *v-myb* sequences) (fig.3).

A 72 bp exon was localized at positions 423–494 from the 3'-end of the Pst174 clone. Both the 5'- and 3'-boundaries of this exon correspond to consensus splice acceptor and donor sequences (AG/GA and CT/GT, respectively).

Another 118 bp exon, also delineated by consensus splice acceptor (AG/CA) and splice donor (AG/GT) sequences, was identified at the 5'-end of the DNA fragment contained in clone Pst049 (see fig.2). The 5'-end of this exon is located 5 bp downstream of the 3'-proximal *Pst*I site (CTGCAG) of EP180.

In order to identify the splice donor sequences from the exon located upstream in the genomic DNA, we took advantage of the fact that a rare restriction site (*Not*I) is located in the 5'-proximal portion of *c-myb* cDNA. Only two *Not*I restriction sites were mapped at the 3'-end of P804 DNA (fig.2), in a 1.0 kb *Sac*I fragment and a 0.9 kb *Sac*I-*Eco*RI fragment. In the vicinity of the *Not*I restriction site, the lymphoma cDNA sequence is missing a 13 bp stretch of nucleotides (CCCCCGTCCTCTT) which is present in both thymic cDNA and genomic DNA (fig.4). The existence of mismatches between the nucleotide se-

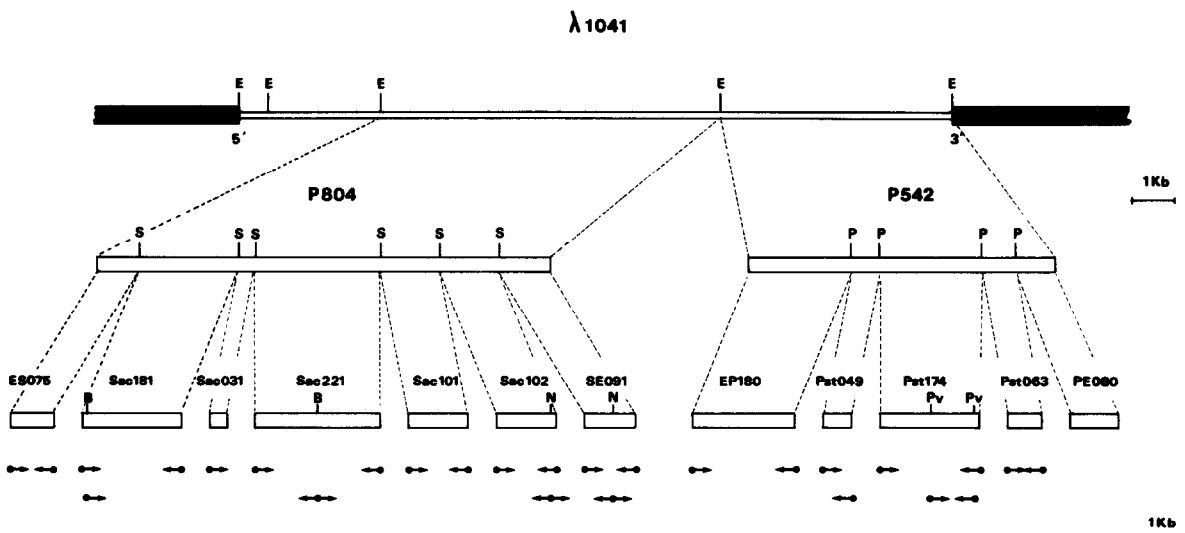


Fig.2. Derivation of DNA clones and sequence strategy used to map *c-myb* exons in chicken DNA. The structure of clone λ1041 has been described [2]. P542 and P804 are subcloned in pBR322 and pUC18, respectively. Only the restriction sites used for subcloning and sequencing (arrows) are indicated. E, *Eco*RI; S, *Sac*I; P, *Pst*I; B, *Bsm*I; N, *Not*I; Pv, *Pvu*II.

quences of lymphoma and thymic cDNAs was already stressed by Gerondakis and Bishop [17]. Although they might result from different experimental procedures used for cDNA cloning and nucleotide sequencing, it is also possible that these differences are related with the various origins of the samples analyzed.

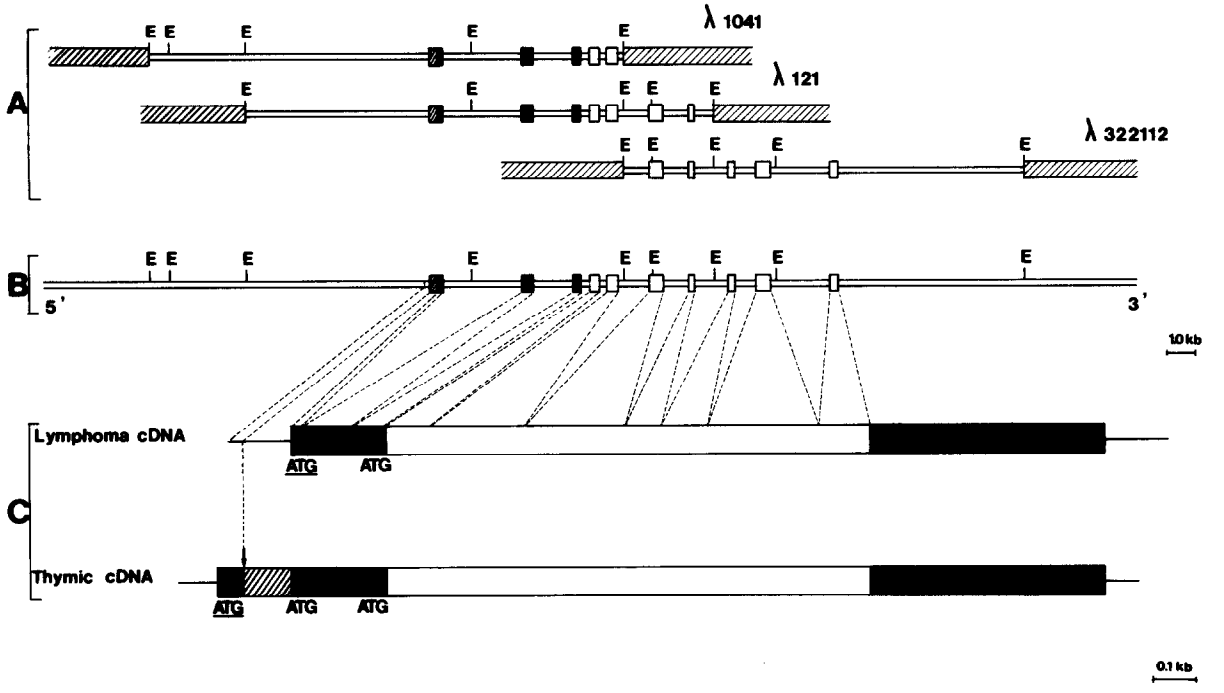
Sequencing performed in both directions from the 3'-proximal *Sac*I site allowed us to show that in this region, both genomic DNA and thymic cDNA are 100% homologous up to a *Sac*II restriction site, where they diverge considerably (fig.4). On the other hand, the 5'-proximal sequence of lymphoma cDNA and genomic DNA are identical upstream to this site. Therefore, the CCTCCC might correspond to the 3'-boundary of an intron which would not be represented in the thymic cDNA, but would be contained in lymphoma cDNA. Interestingly, this sequence does not correspond to a consensus splice acceptor signal. A consensus splice donor sequence (AG/GT) is located 20 nucleotides downstream of the ATG initiation codon, as reported for murine *c-myb* [18]. As discussed below, the ATG codon localized in the 5'-proximal exon that we have mapped corresponds to the *c-myb* initiation codon identified thus far in mouse and human lymphoma cells and to an internal methionine codon in the cDNA isolated from normal thymus cell RNA (figs 3,5). In order to determine whether the leader and 5'-coding sequences found specifically in thymic cDNA were also contained in P804 DNA, we have sequenced several subclones around other restriction sites represented in the 5'-region of this cDNA. Nucleotide sequencing performed in both directions from the *Sac*I and *Bsm*I sites (fig.2) did not allow us to identify any further *c-myb* sequence in P804 DNA.

We have obtained preliminary results indicating that the DNA sequences homologous to the 5'-non-coding region contained in thymic cDNA are located several kilobases upstream of P804.

3.2. c-myb *mRNAs with different 5'-leader sequences are expressed in avian yolk sac and thymic hematopoietic cells*

The nucleotide sequence of the avian *c-myb* cDNAs which have been isolated from thymic cells and B cell lymphoma cells diverge greatly in their 5'-end, while *c-myb* cDNAs isolated from lymphoma cells of different origins show a high degree of homology (fig.1). In order to determine whether these differences result from the tumoral origin of the cDNA, we screened normal RNA species from yolk sac cells for the presence of a leader sequence homologous to that found upstream to the ATG initiation codon identified in thymic cDNA. For this purpose, we isolated the 5'-proximal *Xho*I-*Nco*I fragment containing leader sequences from this cDNA and used it as a probe, after labeling with [α-^{32}P]dCTP. Yolk sac cell RNA electrophoresed on formaldehyde agarose gel and transferred onto nitrocellulose was incubated for hybridization with this probe under the conditions described in section 2. The internal *Nco*I fragment spanning from positions 1 to 453 in the thymic cDNA [15] was used as a positive control. This fragment contains a stretch of 66 nucleotides homologous to the *v-myb* sequences which are also represented in yolk sac RNA. As shown in fig.4, the 4.0 kb *c-myb* RNA species normally expressed in yolk sac cells was detected by the internal *Nco*I

Fig.3. Topography of 5'-proximal *c-myb* exons in chicken DNA. (Top) The organization of the *c-myb* exons in chicken DNA (B) has been drawn from mapping and sequencing performed on the λ recombinant clones whose *Eco*RI (E) restriction maps are shown in (A). Open boxes denote *c-myb* sequences represented in the AMV *v-myb* oncogene, while filled boxes represent *c-myb*-specific exons. (B) The dashed part of the 5'-proximal *c-myb* exon designates a non-coding sequence in lymphoma cDNA and a coding sequence in thymic cDNA (C). A stretch of 22 nucleotides representing leader sequences in lymphoma cDNA (in front of the dashed exon) is probably removed as intron sequences during the maturation of thymic RNA because it is absent in the corresponding cDNA (C). The position of three ATG codons is indicated. From 5' to 3' they correspond to potential translation initiation sites in thymic, lymphoma and yolk sac *c-myb* mRNAs. As shown in fig.5, the thymic and lymphoma cDNA sequences diverge completely upstream to the position marked with an arrow in (C). (Bottom) Nucleotide sequences of *c-myb* exons. E1–E3 represent the 5'-proximal *c-myb*-specific coding sequences found in lymphoma cDNA. E(T) delineates the beginning of additional coding sequences contained in thymic cDNA. Homology between *c-myb* and *v-myb* sequences starts at E4. Intron boundaries are represented by italics. Putative regulatory sequences and translation initiation codons are underlined.



E(T)

.....AACCTGTTTCCTCCTCCTCCTCCCGCGGCTCCTCGGCACTTCGCTGCGCGGCGGCGGAGCGCCTGGGCGCAGCCCGGCAGC

Sac II

E1 E1

AGGGCGCGGCGGCGGGCGCGGGCTCCCCCGTCCTCTTCCCCCAGCGGCCGCCGCGAGGATGGCCCGGAGACCCCGGCACAGGT

Not I

AACGGGGC.....AATACTAGAACAACAGAATGCAGAACAAAATCTTGTTGTGCAAGTTTTAAAACTTTTATCTGTGTAACCTTTGTAA

E2

AGATTGTGTGTTTTCTGTGTGCTGTGTAATTAAGTAGCTCCAGTCCTCACGTTCCGCTCTGTGTCTCGCTGTGTTTCCTGCTGCAGCATATAC

Pst I

AGCAGCGATGACGATGAAGAAGATGTTGAGATGTACGACCACGATTACGACGGCCTGCTTCCTAAGGCTGGGAAACGTCACC

E2 E3

TAGGGAAAACCAGGTGGACCCGTGAAGAGGTAATTGCCG.....AATGATCCAGGATGAGAAACTGAAGAAACTTGTGGAA

E3 E4

CAGAATGGCACAGAAGACTGGAAAGTCATTGCCAGTTTCCTTCCTGTAGGTAGAC.....ATTTCATTAGAATCGGACAGA

TGTTCAGTGCCAG...

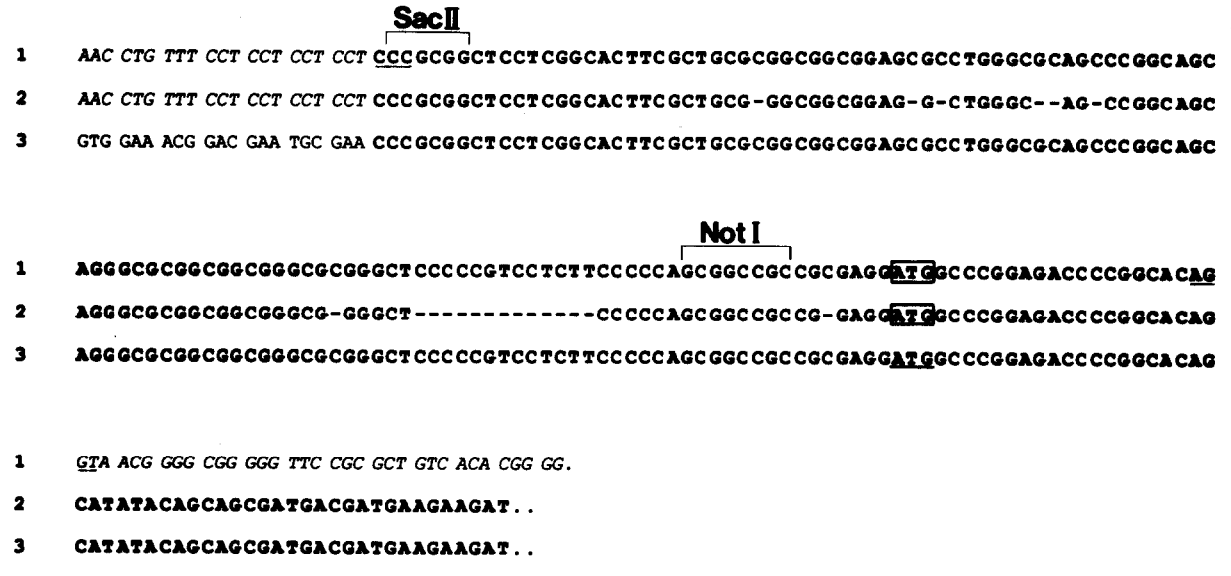
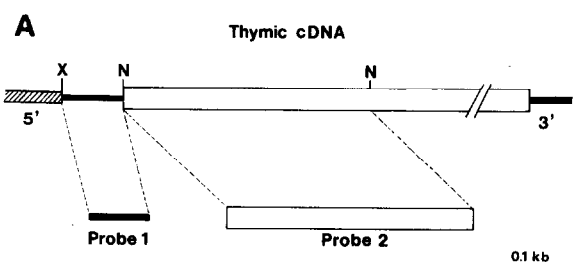


Fig.4. Comparison of avian cDNAs and genomic 5′-proximal *c-myb* sequences. (A) The 5′-proximal sequences of avian lymphoma [2] and thymic [3] *c-myb* cDNAs have been aligned with the *c-myb* nucleotide sequence that we have established in chicken DNA [1]. A perfect matching was obtained between thymic cDNA and genomic sequences downstream of the *Sac*II restriction site. Comparison of the three sequences allows us to locate a non-consensus potential splice acceptor sequence (CCTCCC) in addition to the consensus splice donor signal (AG/GT) located 20 bases downstream of the ATG initiation codon. Sequences homologous to thymic cDNA are indicated by boldface letters. Intron sequences are represented by italics. Note the stretch of 13 nucleotides (CCCCCGTCCTCTT) present in both thymic cDNA and genomic DNA but absent in the lymphoma cDNA.

probe, whereas no hybridization occurred with the 'leader' probe (*Xho*I-*Nco*I). Three other faint bands (3.4, 2.9, 2.5 kb) also detected by the internal *Nco*I probe might correspond to minor *c-myb* species also expressed in yolk sac cells. These results indicate that the sequences located directly upstream to the ATG initiation codon in thymic cDNA are not expressed in yolk sac cells of 13-day-old chicken embryos.



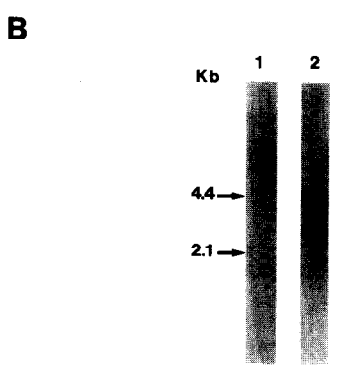


Fig.5. Hybridization of yolk sac cell RNA with *c-myb* cDNA probes. Total RNA was purified from yolk sac cells of 13-day-old chicken embryos under the conditions described in section 2. Two aliquots (10 μg each) of the same RNA preparation were run on a 1% formaldehyde agarose gel and blotted onto nitrocellulose as in [21]. The thymic *c-myb* cDNA previously cloned in Bluescript vector (Rosson, D., personal communication) was digested with *Xho*I and *Nco*I restriction endonucleases. The (*Xho*I-*Nco*I) 110 bp fragment containing thymic leader sequences, and the (*Nco*I-*Nco*I) 450 bp fragment containing the 5′-proximal *c-myb* coding sequences were purified by electrophoresis in polyacrylamide gel, and radiolabeled to be used as probes. (A) Derivation of the probes. (B) Autoradiography of Northern blots hybridized with the leader probe (1) and with the internal *Nco*I fragment (2).

4. DISCUSSION

Several *c-myb* cDNAs have been isolated and sequenced from different sources [7,15,18,19]. All the cDNAs isolated from tumor cells show very high homology in their 5'-coding sequences, independently of their origin (human, mouse or chicken). The isolation of a cDNA corresponding to *c-myb* RNA expressed in normal chicken thymic cells [15] has posed several fundamental problems regarding the regulation of expression of this proto-oncogene. In this cDNA, the ATG triplet, identified in all other cDNAs as a potential initiation codon, encodes for an internal methionine at amino acid position 59 from the N-terminus of the putative corresponding protein (fig.1). Thus, a stretch of 120 nucleotides identified as non-coding leader sequences in chicken lymphoma cDNA appears to encode 40 amino acids of the thymic *c-myb* protein. Upstream of this position (corresponding to amino acid 20 in thymic cDNA, and nucleotide position −120 in lymphoma cDNA) the two sequences diverge completely.

Aside from possible rearrangements occurring in tumor cell DNA, alternative splicing of *c-myb* RNA was proposed to account for these observations [17]. In this case, one additional exon (at least) would be present at the 5'-end of the *c-myb* locus and transcription would proceed from a promoter region located further upstream. Our sequencing data allowed us to localize precisely the boundaries of the 5'-proximal exon containing the ATG initiation codon of lymphoma cDNA, the additional coding sequences represented in thymic cDNA and the putative corresponding intron/exon junction. The potential splice acceptor signal located at this junction does not fall within the general consensus whereas a consensus splice donor sequence (AG/CA) lies 20 nucleotides beyond the ATG codon, as reported in the case of murine *c-myb* cDNA [18]. Several examples of non-consensus donor splice signals have been reported for *c-myb* exons [1] and in the case of a few other chicken genes [24].

A comparison of the genomic nucleotide sequence that we have established with that of thymic and lymphoma cDNAs [15,17] revealed other interesting features regarding the expression of *c-myb* RNA in avian hematopoietic cells. As shown in fig.4 we have found 100% matching between the nucleotide sequences of the lymphoma cDNA leader and the 'intron-exon' junction that we have mapped on the genomic DNA while a high degree of divergence exists at this level between these sequences and that of thymic cDNA. Such a variation in the 5'-proximal structure of two *c-myb* cDNAs most probably reflects tissue-specific initiation of transcription, rather than solely alternative splicing process. This would account for the different structure of leader sequences in thymic and yolk sac *c-myb* RNA (fig.5). The lack of hybridization between yolk sac *c-myb* RNA and P804 DNA also raised another interesting question, since we have mapped in this fragment 119 nucleotides represented in lymphoma cDNA (fig.4). Furthermore, preliminary results obtained in mapping the 5'-terminus of yolk sac *c-myb* mRNA (not shown) were consistent with the presence of a promoter region at the 5'-end of P504 DNA. Interestingly, an ATG codon, located at position 38 in the Pst049 *c-myb* exon (fig.3) appears to be in phase with the remainder of the *c-myb* protein, and sequencing of DNA upstream of this exon allowed us to recognize both a potential TATA box (TAATTAA) and a −80 region (GAACAAAA) at positions 59 and 135, respectively, from the 3'-end of EP180 DNA.

To explain all these observations, we propose that different promoter regions are used to initiate *c-myb* transcription in hematopoietic cells of different origins. Our preliminary results indicate that the genomic sequences homologous to the thymic cDNA leader are located several kilobases upstream to the exon containing the ATG initiation codon found in lymphoma cDNA. This model would also account for the expression of more than one *c-myb* RNA in hematopoietic cells ([25]; unpublished) and would be in agreement with previous reports suggesting that transcription of the murine *c-myb* locus might be initiated at multiple sites [6,26]. Alternative splicing has already been associated with tissue-specific expression of mRNA [27] and a shift in promoter utilisation is well documented in the case of the *c-myc* gene [28–31]. More recently, it has been shown that transcription of the NMYC locus also initiates at numerous sites that may be under the control of two promoters and involves alternative splicing [32]. Experiments are now in progress to establish the precise organization of the regulatory se-

quences governing the tissue-specific expression of *c-myb* and to determine whether the expression of different *c-myb* mRNAs can be correlated with particular stages of hematopoiesis and tumor genesis.

Acknowledgements: This work was supported by grants from the Centre National de la Recherche Scientifique, Association pour la Recherche sur le Cancer, Fondation contre la Leucémie, and Fondation pour la Recherche Médicale. We are grateful to Drs D. Rosson and E.P. Reddy for providing their *c-myb* cDNA clone.

REFERENCES

[1] Klempnauer, K.H., Gonda, T.J. and Bishop, J.M. (1982) Cell 31, 453–463.
[2] Perbal, B., Cline, J., Hillyard, R.L. and Baluda, M.A. (1983) J. Vjrol. 45, 925–940.
[3] Boyle, W.J., Lipsick, S.J., Reddy, E.P. and Baluda, M.A. (1983) Proc. Natl. Acad. Sci. USA 80, 2834–2838.
[4] Klempnauer, K.H., Ramsay, G., Bishop, J.M., Moscovici, M.G., Moscovici, C., McGrath, J.P. and Levinson, A.D. (1983) Cell 33, 345–355.
[5] Klempnauer, K.H., Symonds, G., Evan, G.I. and Bishop, M.J. (1984) Cell 37, 537–547.
[6] Leprince, D., Saule, S., De Taisne, C., Gegonne, A., Begue, A., Rocjo, M. and Stehelin, D. (1983) EMBO J. 2, 1073–1078.
[7] Gonda, T.J., Gough, N.M., Dunn, A.R. and De Blaquiere, J. (1985) EMBO J. 4, 2003–2008.
[8] Katzen, A.L., Kornberg, T.B. and Bishop, J.M. (1985) Cell 41, 449–456.
[9] Torelli, G., Selleri, L., Donnelli, A., Ferrari, S., Emilia, G., Venturelli, D., Moretti, L. and Torelli, U. (1985) Mol. Cell. Biol. 5, 2874–2877.
[10] Reed, J.C., Alpers, J.D., Nowell, P.C. and Hoover, R.G. (1986) Proc. Natl. Acad. Sci. USA 83, 3982–3986.
[11] Stern, J.B. and Smith, K.A. (1986) Science 233, 203–206.
[12] Gonda, T.J., Sheiness, D.K. and Bishop, J.M. (1982) Mol. Cell. Biol. 2, 617–623.
[13] Westin, E.H., Gallo, R.C., Arya, S.K., Souza, L.M., Baluda, M.A., Aaronson, S.A. and Wong-Staal, F. (1982) Proc. Natl. Acad. Sci. USA 79, 2194–2198.
[14] Thompson, C.B., Challoner, P.B., Neiman, P.E. and Groudine, M. (1986) Nature 319, 474–480.
[15] Rosson, D. and Reddy, E.P. (1986) Nature 319, 604–606.
[16] Romanoff, A. (1960) The Avian Embryo, Macmillan, New York.
[17] Gerondakis, S. and Bishop, J.M. (1986) Mol. Cell. Biol. 6, 3677–3684.
[18] Bender, T.P. and Kuehl, W.M. (1986) Proc. Natl. Acad. Sci. USA 83, 3204–3208.
[19] Majello, B., Kenyon, L.C. and Dalla-Favera, R. (1986) Proc. Natl. Acad. Sci. USA 83, 9636–9640.
[20] Baluda, M.A. and Goetz, I.E. (1961) Virology 15, 185–189.
[21] Perbal, B. (1988) A Practical Guide to Molecular Cloning, 2nd edn, Wiley, New York.
[22] Perbal, B. and Baluda, M.A. (1982) J. Virol. 41, 250–257.
[23] Perbal, B. and Baluda, M.A. (1982) J. Virol. 44, 586–594.
[24] Mount, S.M. (1982) Nucleic Acids Res. 10, 459–472.
[25] Franchini, G., Wong-Staal, F., Baluda, M.A., Lengel, C. and Tronick, S.R. (1983) Proc. Natl. Acad. Sci. USA 80, 7385–7389.
[26] Watson, R.T.J., Dyson, P.J. and McMahon, J. (1987) EMBO J. 6, 1643–1651.
[27] Stanton, L.W., Watt, R. and Marcu, K.B. (1983) Nature 303, 401–406.
[28] Yang, J., Bauer, S.R., Mushinski, J.F. and Marcu, K.B. (1985) EMBO J. 4, 1441–1447.
[29] Reicin, A., Yang, J., Marcu, K.B., Fleissner, E., Koehne, C.F. and O'Donnell, P.V. (1986) Mol. Cell. Biol. 6, 4088–4092.
[30] Broome, H.E., Reed, J.C., Godillot, E.P. and Hoover, R.G. (1987) Mol. Cell. Biol. 7, 2988–2993.
[31] Stanton, L.W. and Bishop, J.M. (1987) Mol. Cell. Biol. 7, 4266–4272.