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QUARTERLY

Cloning and characterization of the 5′ part of the high molecular weight transcript of rat DNA polymerase β*

Ryszard Konopiński, Radosława Nowak and Janusz A. Siedlecki

**Department of Cell Biology and Experimental Therapy, Cancer Center of the M. Skłodowska-Curie Institute, Wawelska 15, 02–034 Warsaw, Poland*

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A rat brain cDNA library has been constructed. Three identical clones of about 2.2 kb representing high molecular weight DNA polymerase β transcript were found. Sequencing proved our earlier suggestion that both high and low molecular weight DNA polymerase β transcripts have the same open reading frame and differ mainly at the 3′ end. Because of that, alternative polyadenylation is discussed as a possible mechanism for tissue- and development-specific regulation of the DNA polymerase β gene expression.

DNA polymerase β (β-pol) is one of the five known vertebrate cellular DNA polymerases [1–5]. The enzyme is present in all tissues but its level is relatively low and varies only slightly during the cell cycle [6, 7]. The structure of the DNA polymerase β is strongly conserved throughout evolution [8]. This suggests the importance of this enzyme for maintenance of the cell. One of the main functions suggested for DNA polymerase β is filling of DNA nicks and gaps during DNA excision repair [2, 3, 9, 10]. The enzyme is also involved in post-recombinational DNA synthesis [11–13]. Thus, it is an important enzymatic component involved in meiosis and recombination DNA repair.

Studies on the β-polymerase gene in humans and rats have shown that the enzyme is specified by a single-copy gene. cDNAs for rat and human DNA polymerase β have been cloned [14]. Studies on β-polymerase gene expression in rat revealed the presence of at least two major transcripts (1.4 and 4.0 kb). Our previous data strongly suggest that this expression is mainly regulated at the mRNA level [15]. In addition tissue specific gene expression has been shown [12, 13, 15]: the relative amounts of the two transcripts differ in different tissues. For example, in testis the level of the 1.4 kb transcript is almost 15 times higher as compared to that of the 4.0 kb transcript whereas in 7-day old brain the level of the 4.0 kb transcript is about 5 times higher than that of the 1.4 kb transcript. Cells with the two different mRNA types produce only one type of protein molecule. This suggests differences between the two types of β-pol mRNA in the 3′ and 5′ non-coding regions of the molecule, but not in the open reading frame (ORF). Full determination of the structural differences between the two mRNAs could allow us to describe another molecular mechanism of DNA polymerase β gene expression. The molecular structure of the shorter (1.4 kb) transcript has been previously established [14]. The presented here detailed ana-

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**Present address: Department of Molecular Biology, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, P. Findera 101, 02–784 Warsaw, Poland

lysis of the clone containing a part of the high molecular weight transcript confirms that both transcripts have the same ORF. The difference is at the 3′ end, probably because of alternative polyadenylation. The possible role of alternative polyadenylation in tissue and development specific gene expression is discussed.

MATERIALS AND METHODS

Animals. Male albino rats (Wistar) were maintained at room temperature and provided with tap water and laboratory chow *ad libitum*. Organs obtained from rats were immediately frozen in liquid nitrogen and stored at –70°C.

cDNA library construction and screening. mRNA used for cDNA synthesis was isolated from seven-day-old rat brains. As we have shown previously [13] this tissue has the highest level of 4.0 kb transcript. Total RNA was prepared using the guanidinium isothiocyanate procedure according to Chomczynski & Sacchi [16]. Poly(A)$^+$mRNA was isolated by a double passage through an oligo(dT) column. The cDNA synthesis was carried out using Amersham's "cDNA Synthesis System Plus" with cDNA methylation as *Eco*RI sites protection and oligo(dT) as a primer. cDNA was then size fractionated by agarose gel electrophoresis. Only fragments larger than 1.5 kb were taken for cloning. Vector λ_{gt11} DNA and synthetic *Eco*RI linkers were used as the cloning system. cDNA library was screened by plaque hybridization according to Maniatis *et al.* [17]

Molecular probes. Plasmid inserts containing the 3′ or 5′ part of the short transcript polymerase β cDNA were used as molecular probes. These probes were kindly gifted by Dr. B. Żmudzka. The restriction map of this cDNA and the names of the probes are shown in Fig. 1. Inserts were radiolabelled by the "random priming" method using [α-^{32}P]dCTP. The average specific activity was 5 × 10^8 c.p.m./μg DNA.

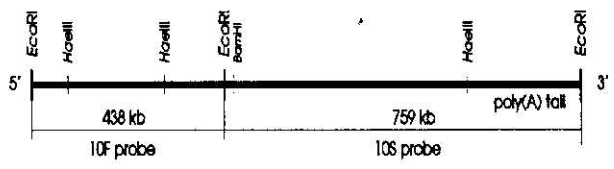


Fig. 1. The restriction map of the 1.4 kb rat transcript cDNA showing fragments used as the probes.

Northern analysis. Poly(A)$^+$RNAs (10 μg) were electrophoresed overnight at 18 V and, after denaturation according to Maniatis *et al.* [17], the gels were blotted onto nitrocellulose. Prehybridization and hybridization were carried out at 42°C with 50% formamide as published previously [11]. As molecular radiolabelled probes 10F and 10S fragments (Fig. 1) or DNA inserts from cloned cDNA were used.

pUC19 subcloning. Isolated bacteriophage DNA was digested with restriction endonuclease *Eco*RI. Restriction fragments were separated on agarose gels and then inserts were isolated using NA-45 Schleicher & Schull DEAE-cellulose. After elution from the membrane, inserts were ligated with the *Eco*RI digested and dephosphorylated pUC19 plasmid vector [17].

DNA sequencing and oligonucleotide synthesis. DNA sequencing was carried out by the standard Sanger enzymatic method with T7 DNA polymerase. Sequencing reactions were primed with commercial universal pUC primers or newly synthesized oligonucleotides. Chemical oligonucleotide syntheses were carried out with Applied Biosystems 391 DNA synthesizer.

RESULTS

Three clones with homology to both 3′ (10S probe) and 5′ (10F probe) parts of the 1.4 kb transcript of β polymerase have been identified and isolated from a cDNA library. All three clones carried inserts of about the same length (approximately 2.2 kb). The 2.2 kb inserts had two internal *Eco*RI sites, dividing them into parts of 0.45, 0.65 and 1.1 kb in length. These parts have been named S, M and L, respectively. Restriction analysis with *Hae*III and *Sau*3AI endonucleases confirmed that all three clones were identical (not shown).

The 2.2 kb cDNA was further analysed by partial digestion with *Eco*RI and hybridization with 10S and 10F β-pol probes (Fig. 2A). Six bands are observed on the gel (2.2 kb, 1.75 kb, 1.55 kb, 1.1 kb, 0.65 kb, 0.45 kb). Probe 10S hybridized to four of them (2.2 kb, 1.75 kb, 1.55 kb, 1.1 kb), whereas probe 10F hybridized only

to three (2.2 kb, 1.55 kb, 0.45 kb). These results as well as the results of complete *Eco*RI digestion allow us to map the relative position of fragments S, L and M (Fig. 2B).

According to this map fragment M should hybridize only to the high molecular weight transcript (4.0 kb) of β-polymerase. Indeed, when fragment M was used as a molecular probe, only the hybridization with the 4.0 kb transcript was observed. Hybridization to the 1.4 kb transcript was not detected either in RNA preparations from the brain or from the testis in which the amount of this transcript is more than 15 times as high as in the brain (Fig. 3). Fragments L and S hybridized both to low and high molecular weight transcript (data not shown). Additionally, S hybridized only to 10F fragment whereas L hybridized only to 10S (Fig. 2A).

Fragments S, M and L have been independently subcloned into the pUC19 vector. Thus, three pairs of pUC clones have been obtained with plasmids containing inserts in both orientations in each pair. The complete nucleotide sequence from both strands of all three fragments has been obtained. As shown in Fig. 4, the 2.2 kb insert contains a long open reading frame initiating with an ATG at nucleotide positions 59–61 and terminating with a TGA stop codon at positions 1013–1015, followed by the 3′ noncoding region. Comparison of the obtained sequence with the sequence of the 1.4 kb transcript revealed that the region 17–1155 is identical to the complete sequence of the short transcript excluding the poly(A) tail at its 3′ end. The first sixteen nucleotides on the 5′ end of the cloned cDNA are absent in the sequence of the cDNA of the shorter transcript, but this fragment has been found in the sequence of the related mouse polymerase-β gene promoter region at its positions 45–60 [18].

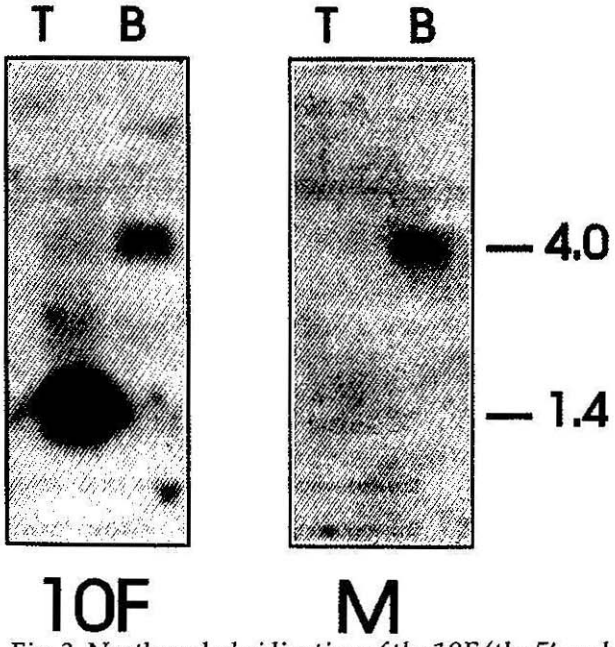


Fig. 3. Northern hybridization of the 10F (the 5′-end fragment of the pol-β ORF) and M probes to the poly(A)$^+$ RNA isolated from testis (lane T) and 7-day old rat brain (lane B).

DISCUSSION

The clones isolated from the rat brain cDNA library correspond to the fragment of the high-molecular weight transcript of DNA polymer-

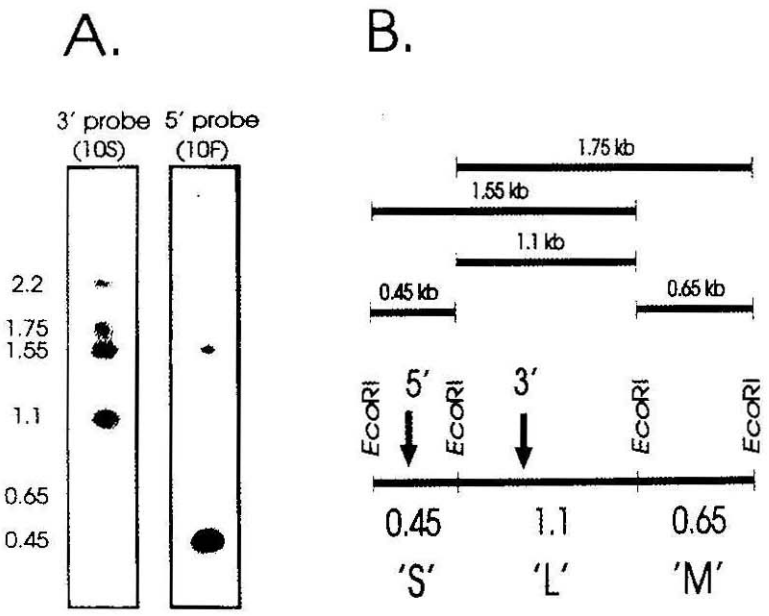
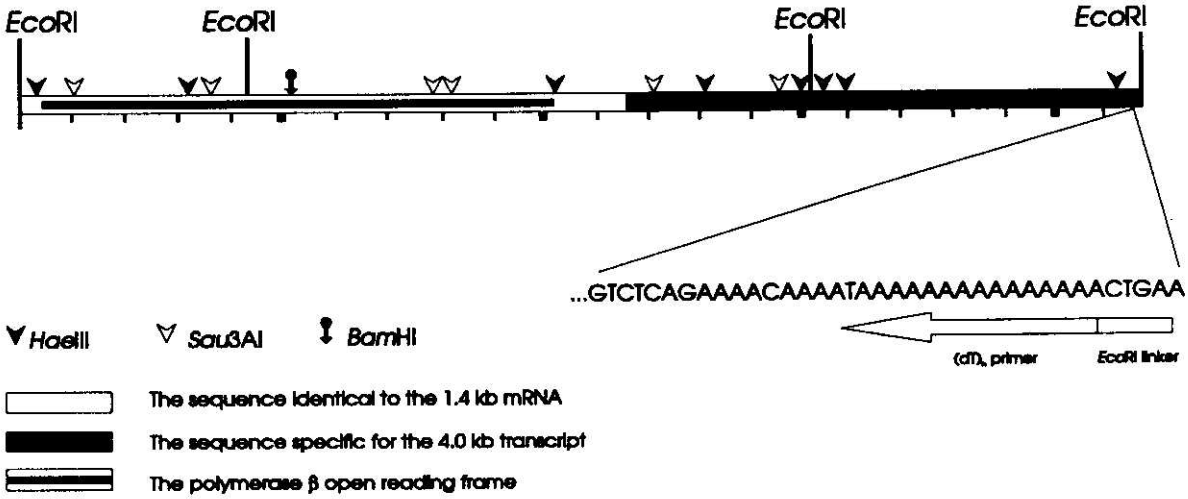


Fig. 2. Southern blot analysis of 2.2 kb β-pol cDNA.
cDNA was partially digested with *Eco*RI as described in Materials and Methods. The DNA was then electrophoresed, blotted onto nitrocellulose and hybridized to the 10F and 10S probes (A). The *Eco*RI restriction map of the cloned cDNA and designation of fragments are shown on Fig. 2B.



cggcaccatg agcaaaCGCA AGGCCCCGCA GGAGACCCTC AACGGCGGCA TCACGGACAT GCTCGTGGAA CTCGCAAACT
TTGAGAAGAA CGTGAGCCAG GCGATCCACA AGTACAATGC ATACAGAAAA GCAGCATCTG TGATAGCAAA GTACCCACAC
AAAATCAAGA GTGGAGCAGA AGCTAAGAAA TTGCCAGGAG TAGGAACAAA AATTGCTGAA AAGATTGATG AATTTTTAGC
AACTGGAAAA TTGCGTAAAC TGGAAAAGAT TCGTCAGGAT GATACAAGTT CATCCATCAA CTTCCTGACT CGAGTTACTG
GCATCGGGCC ATCTGCTGCA AGGAAGCTTG TAGATGAAGG AATTAAAACA TTAGAAGATC TCAGGAAAAA TGAAGATAAA
TTGAACCACC ATCAGCGAAT TGGGCTGAAA TATTTTGAGG ACTTTGAAAA GAGAATTCCT CGTGAGGAGA TGCTGCAAAT
GCAGGACATT GTTCTTAATG AAGTTAAAAA GCTGGATCCC GAGTACATCG CTACAGTCTG CGGCAGTTTC CGAAGAGGCG
CAGAGTCCAG TGGAGATATG GACGTTCTGC TGACCCACCC AAACTTCACG TCAGAATCAA GCAAACAGCC AAAGTTGTTA
CATCGTGTTG TGGAACAGTT ACAAAAAGTC CGTTTCATTA CAGATACTCG TTCAAAGGGT GAGACAAAGT TCATGGGTGT
TTGCCAGCTT CCCAGCGAGA ATGATGAAAA CGAATATCCA CACAGGAGAA TCGATATCAG GTTGATCCCC AAAGATCAGT
ACTACTGTGG TGTTCTCTAC TTCACTGGAA GTGACATCTT TAATAAGAAT ATGAGAGCGC ATGCCCTGGA AAAGGGCTTC
ACAATCAATG AGTACACCAT CCGCCCCCTG GGGGTCACTG GGGTTGCTGG GGAGCCCCTT CCGGTGGACA GCGAGCAGGA
CATTTTCGAT TACATCCAGT GGCGCTACCG GGAGCCCAAG GACAGGAGTG AATGACGCCT GCCCCACCCA GGCCTATCCC
ACGAGGAGTC CTAAGTTATT GCTTAACCTT TGCTATGTAA GGGTTGGGTG TTTTAGGTGA TTGCCTCTTC TCTGTGCTTC
CCATGGACTA TCGTC**AATGA** **A**CCTCTTGAG CAGCTattca gtattttctt agcacatgtg tttaaactta gatatgtttg
tgatcgcagt accatggttt actagagaaa cgtaagagcc gatgtgataa gcagtgttta atgacctttc acttccacct
cacattgcag ccacagtcgg ccccacttgt ccctatccac cctttatggg tcagctggtc tcccagagcc atctccagaa
gtcacttcat tatccttaga aacaaggggc ttccaggcat ggtggtgcac atccttaatc ccaggacttg ggaggcagag
acaggcagat ctctgtgagt tcaaggccag cccagactac atactgaatt cagggtgcca gagctacata gtgaggccct
atctaaaaag aaaggggcct tcatagctgt aagacttctg tgaccatttc ccatttgcag tgtatactga taactagaag
acagtgtgtg ctttacctca ggggcagcct tcccctcaca gctcaatggc acctaactag cattcatcag tcgtctggtg
gacagtccac tgtttatcaa ataacattct gtgacacgaa caaagaactt caagtttaag aaagatttat ttgagcagat
tgagccactg tgtctggatg gctttattgt agagtcctgt catgttgtag atggtagtca caaagtcaga agtaactcat
cacagcgtct ggtcactgtc agttactaga agattcagct ttatttatgt gctgggtata atgtacttgc ctgtaatccc
aagactcact cgaggcaaga ggaacgtgag ttcgaagccc accctgggct atatagctta ctctgagacc ctgtctcaga
acccttaccc ctccccccaa aaattatcca ggaggcagag gcaggtgggt ctctatgggt gtaaggccat ccagggctac
acagcgagac cactgtctca gaaaacaaaa taaaaaaaaa aaaaaactga attc

Fig. 4. The restriction map and the nucleotide sequence of the cloned cDNA fragment of the high-molecular weight polymerase β transcript.
Capital letters indicate the sequence identical to the sequence of 1.4 kb transcript. The open reading frame is underlined. Boldface letters indicate the probable polyadenylation signal for the 1.4 kb transcript.

ase β. This conclusion is based on the following observations:

- 1. The total length of the cloned cDNA (2.2 kb) is considerably greater than 1.4 kb — the maximal length value for the shorter polymerase-β transcript.
- 2. The fragment M of the cloned cDNA hybridizes to 4.0 kb mRNA of DNA polymerase β, but it does not hybridize to the 1.4 kb transcript.
- 3. The length of the fragment L is by about 300 bp greater than the related short transcript *Eco*RI fragment (10S).

The sequence analysis of the 2.2 kb cDNA revealed that both transcripts have the same ORF and a part of 3′ noncoding sequences. The further sequence of fragment L and the whole fragment M corresponds to high-molecular weight DNA polymerase β transcript. The presence of the A-rich region at the 3′ end of the 2.2 kb cDNA (Fig. 4) could be a reason for the repeating synthesis of the shorter than full-length (about 4 kb) cDNA [19].

The pre-mRNA polyadenylation site has been precisely determined in animal cells [20–23]. The hnRNA cleavage and polyadenylation events are directed by the sequence elements located upstream and downstream of the cleavage site. The most important element, AAUAAA, is typically located 10–35 nucleotides upstream of the poly(A) site. Sequence analysis of the 1.4 kb transcript revealed an AAUGAA sequence 20 nucleotides upstream of the 1.4 kb transcript polyadenylation site with one mismatching nucleotide (G instead of A). This sequence may serve as a weak polyadenylation signal. Therefore, we suggest that the high molecular weight transcript is generated by alternative polyadenylation.

We have demonstrated previously the tissue- and development-specific β-polymerase gene expression [11, 13, 15]. The precise mechanism of such regulation remains unexplained. It was shown that certain DNA polymerase β gene sequences could act as the tissue- and development-specific regulatory elements [24, 25]. On the other hand, there are some experimental data which suggest involvement of the alternative polyadenylation in regulation of other genes [23], particularly in their tissue- and development-specific expression [26–28]. The possible role of alternative polyadenylation in regulation of the DNA polymerase β gene expression could be explained by the different stability of the two messengers with different 3′ non-coding regions. There are examples of the selective degradation of mRNAs containing defined sequence elements in their 3′ non-coding regions [29–31]. On the other hand, it is possible that certain sequences responsible for higher structure formation could stabilize the mRNA molecule. In the cloned cDNA fragment of the high molecular weight transcript of DNA polymerase β there are regions with sequences suggesting their involvement in such interactions. These are long G and A-rich tracts and possible hairpin loop-forming palindromes.

REFERENCES

1. Wang, T.S.-F. (1991) Eucaryotic DNA polymerases. *Annu. Rev. Biochem.* **60**, 513–552.
2. Perrino, F.W. & Loeb, L.A. (1990) Animal cell DNA polymerases in DNA repair. *Mutation Res.* **236**, 289–300.
3. Wilson, S., Abbotts, J. & Widen, S. (1988) Progress toward molecular biology of DNA polymerase β. *Biochim. Biophys. Acta* **949**, 149–157.
4. Fry, M. & Loeb, L.A. (1986) *Animal Cell DNA Polymerases*. CRC Press, Boca Raton, Fl.
5. Bambara, R.A. & Jessee, C.B. (1991) Properties of DNA polymerases δ and ε and their roles in eucaryotic DNA replication. *Biochim. Biophys. Acta* **1088**, 11–24.
6. Spadari, S. & Weissbach, A.J. (1974) The Interrelation between DNA synthesis and various DNA polymerase activities in synchronized HeLa cells. *Mol. Biol.* **86**, 11–20.
7. Chiu, R.W. & Baril, E.F. (1975) Nuclear DNA polymerases and the HeLa cell cycle. *J. Biol. Chem.* **250**, 7951–7957.
8. Chang, L.M.S., Plevani, P. & Bollum, F.J. (1982) Evolutionary conservation of DNA polymerase β structure. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 758–761.
9. Nowak, R., Kulik, J. & Siedlecki, J.A. (1987) The ability of DNA polymerase β to synthesize DNA beyond the gap with displacement of the non-replicated strand. *Acta Biochim. Polon.* **34**, 205–215.
10. Singhal, R.K. & Wilson, S.H. (1993) Short gap-filling synthesis by DNA polymerase β is processive. *J. Biol. Chem.* **268**, 15906–15911.
11. Nowak, R. & Siedlecki, J.A. (1991) Effect of busulphan treatment and elevated temperature

on the expression of the β-pol gene in rat testis. *Mol. Biol. Reports* **15**, 25–31.

12. Hirose, F., Hotta, Y., Yamaguchi, M. & Matsukage, A. (1989) Difference in the expression level of DNA polymerase β among mouse tissues: high expression in the pachytene spermatocyte. *Exp. Cell Res.* **181**, 169–180.
13. Nowak, R., Woszczynski, M. & Siedlecki, J.A. (1990) Changes in the DNA polymerase β gene expression during development of lung, brain, and testis suggest an involvement of the enzyme in DNA recombination. *Exp. Cell Res.* **191**, 51–56.
14. Zmudzka, B.Z., SenGupta, D., Matsukage, A., Cobianchi, F., Kumar, P. & Wilson, S.H. (1986) Structure of rat DNA polymerase β revealed by partial amino acid sequencing and cDNA cloning. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5106–5110.
15. Nowak, R., Siedlecki, J.A., Kaczmarek, L., Zmudzka, B.Z. & Wilson, S.H. (1989) Levels and size complexity of DNA polymerase β mRNA in rat regenerating liver and other organs. *Biochim. Biophys. Acta* **1008**, 203–207.
16. Chomczynski, P. & Sacchi, N. (1987) Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156–159.
17. Maniatis, T., Fritsch, E.F. & Sambrook, J. (1989) *Molecular cloning. A Laboratory Manual.* Cold Spring Harbor, N.Y.
18. Yamaguchi, M., Hirose, F., Hayashi, Y., Nishimoto, Y. & Matsukage, A. (1987) Murine DNA polymerase β gene: mapping of transcription initiation sites and the nucleotide sequence of the putative promoter region. *Mol. Cell Biol.* **7**, 2012–2018.
19. Frohman, M.A., Dush, M.K. & Martin, G.R. (1988) Rapid production of full-length cDNAs from rare transcripts: Amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8998–9002.
20. Wickens, M. (1990) How the messenger got its tail: Addition of poly(A) in the nucleus. *Trends Biochem. Sci.* **15**, 277–281.
21. Proudfoot, N. (1991) Poly(A) signals. *Cell* **64**, 671–674.
22. Wahle, E. & Keller, W. (1992) The biochemistry of 3′-end cleavage and polyadenylation of messenger RNA precursors. *Annu. Rev. Biochem.* **61**, 419–440.
23. Leff, S.E., Rosenfeld, M.G. & Evans, R.M. (1986) Complex transcriptional units: Diversity in gene expression by alternative RNA processing. *Annu. Rev. Biochem.* **55**, 1091–1117.
24. Yamaguchi, M., Hayashi, Y., Furukawa, K., Nishimoto, Y. & Matsukage, A. (1991) Organization of mouse DNA polymerase β gene silencer elements and identification of the silencer-binding factor(s). *Jpn. J. Cancer Res.* **82**, 72–81.
25. Widen, S.G. & Wilson, S.H. (1991) Mammalian β-polymerase promoter: large-scale purification and properties of ATF/CREB palindrome binding protein from bovine testes. *Biochemistry* **30**, 6296–6305.
26. Wang., Y.-C. & Rubenstein, P.A. (1992) Choice of 3′ cleavage/polyadenylation site in β-tropomyosin RNA processing is differentiation-dependent in mouse BC_3H_1 muscle cells. *J. Biol. Chem.* **267**, 2728–2736.
27. Thekkumkara, T.J., Livingston, W.III., Kumar, R.S. & Sen, G.C. (1992) Use of alternative polyadenylation sites for tissue-specific transcription of two angiotensin-converting enzyme mRNAs. *Nucleic Acids Res.* **20**, 683–687.
28. De Jaegere, S., Wuytack, F., De Smedt, H., Van Den Bosch, L. & Casteels, R. (1993) Alternative processing of the gene transcripts encoding a plasma-membrane and a sarco/endoplasmic reticulum Ca^{2+} pump during differentiation of BC_3H_1 muscle cells. *Biochim. Biophys. Acta* **1173**, 188–194.
29. Foulkes, N.S., Schlotter, F., Pevet, P. & Sassone-Corsi, P. (1993) Pituitary hormone FSH directs the CREM functional switch during spermatogenesis. *Nature (London)* **362**, 264–267.
30. Shaw, G. & Kamen, R. (1986) A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* **46**, 659–667.
31. Asson-Batres, M.A., Spurgeon, S.L., Diaz, J., DeLoughery, T.G. & Bagby, G.C., Jr. (1994) Evolutionary conservation of the AU-rich 3′ untranslated region of messenger RNA. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1318–1322.