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Presence of multiple functional polyadenylation signals in the 3′-untranslated region of human corticotropin receptor cDNA

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Abstract

We present 2.59 kb of the 3′-non-coding region of the ACTH receptor cDNA that contains seven potential polyadenylation signals. Among these signals, five are functional as detected by 3′-RACE and are consistent with the transcripts of 1.8, 3.4 and 4 kb visualized on Northern blots. We propose that the most likely molecular mechanism for the multiple ACTH-R mRNA transcripts is the alternative use of polyadenylation signals.

Keywords: ACTH receptor, human; 3′-Untranslated region; Polyadenylation site

The cloning of the human ACTH and MSH receptor cDNAs has demonstrated that the ACTH receptor (ACTH-R or MC2-R) is a member of the superfamily of G protein-coupled receptors with seven hydrophobic transmembrane domains [1,2]. The ACTHR is predicted to be 297 amino-acids long, making it the smallest known G protein-coupled receptor, resulting from short extracellular N- and intracellular C-terminal domains and from a small second extracellular loop [3,4]. Moreover, this receptor lacks several amino-acid residues present in most G protein-coupled receptors [5]. These unusual structural features of the ACTH-R set it apart in a unique family of G protein-coupled receptors including MSH receptors.

The presence of several transcripts coding for the ACTH-R has been reported in cultured human and bovine adrenocortical cells [6,7] as well as in human and mouse adrenocortical cell lines [8]. Since the entire coding sequence is contained in a single exon [1] and since one major transcription start site is used for the human ACTH-R gene [9,10], the presence of transcripts of different sizes could result from the use of alternative polyadenylation signals. Poly(A) signals in higher eukaryotes are well-defined. Highly conserved RNA sequences consist of two parts, the hexamer AAUAAA located 10–30 nucleotides upstream of the cleavage site and a GU- or U-rich downstream element [11–13]. Typically, poly(A) signals are found in the 3′-untranslated region (3′-UTR) of RNA transcripts. Moreover, the 3′-UTR is thought to influence important characteristics of mRNA including transport, stability, translational efficiency and gene expression [12–15]. So, the characterization of the 3′-UTR of cDNAs is of great interest and we report here the 3′-nucleotide sequence of the major transcripts of the human ACTH-R.

Northern blot analysis of human ACTH-R mRNA revealed the presence of at least two major transcripts of 1.8 and 3.4 kb and three minor ones of 4, 7 and 11 kb (Fig. 1). Moreover, the 1.8 kb band was large

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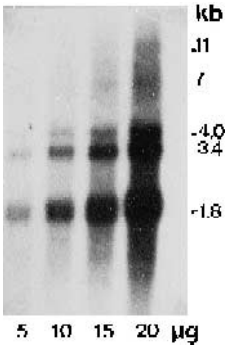

Fig. 1. Northern blot analysis of human ACTH-R mRNAs. Total RNA was extracted from human adrenal gland. Increasing amounts of RNA were loaded onto the gel and the Northern blot was hybridized with a cDNA probe corresponding to the entire coding sequence of the human ACTH-R gene [6].

even with less RNA loaded and could account for more than one transcript. So, we decided to characterize the 3′-UTR of the human ACTH-R cDNA to detect functional poly(A) signals. Rapid amplification of cDNA ends (RACE) was performed after synthesis of a first strand cDNA using 1 μg poly(A^+) mRNAs extracted from human adrenal gland [16]. Using the 3′-RACE procedure, two cDNAs (PCR products A and B) containing unreported 3′-UTR were amplified using oligonucleotides 1 and 2 specific of the coding region of the ACTH-R gene and the anchor primer (Table 1, Fig. 2). Each cDNA contained a poly(A) signal (AATAAA) at positions 1404 for PCR product A and 1578 for PCR product B followed by poly(A) tails 19 and 21 bases downstream, respectively (Fig. 3). In addition, a poly(A) signal at position 1232 was not followed by a poly(A) tail. Since another major transcript of 3.4 kb was observed on Northern blot, we performed another 3′-RACE with oligonucleotides 3 and 4 as specific primers, chosen in the 3′-UTR of the ACTH-R cDNA previously characterized and the same anchor primer. Two cDNAs (PCR products C and D) were amplified and three poly(A) signals

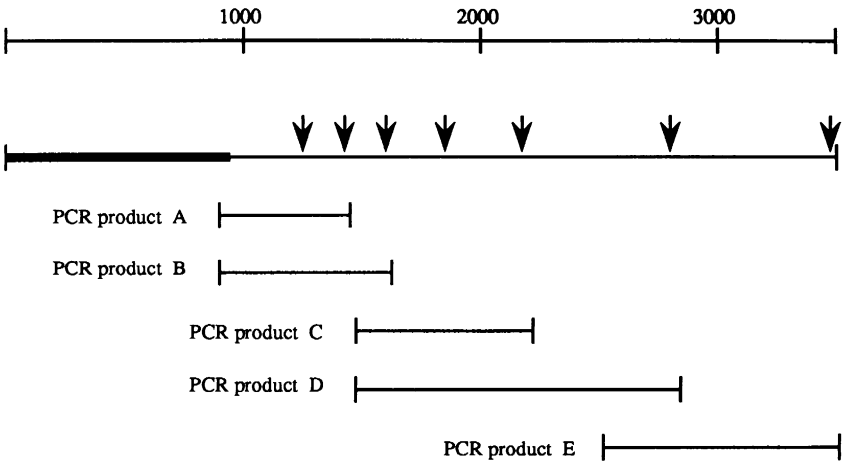

Fig. 2. Schematic diagram of the human ACTH-R cDNA 3′UTR obtained by 3′RACE. The first cDNA strand was synthesized using 1 μg A^+)mRNAs extracted from human adrenal gland and an oligo(dT) cDNA synthesis primer containing an anchor sequence (5′-CCTCTGAAGGTTCCAGAATCGATAGGAATTCTTTTTTTTTTTTTTTT(GCA)(GCAT)-3′) (Clontech, Ozyme, Montigny-le Bretonneux, France). A primary PCR reaction was performed using two primers: one is the anchor primer (5′-CTGGTTCGGCCCAC-CTCTGAAGGTTCCAGAATCGATAG-3′) that anneals to the anchor sequence incorporated during cDNA synthesis, the other primer was specific of the human ACTH-R cDNA (oligonucleotide 1, Table 1). To ensure maximum specificity, an aliquot of the primary PCR reaction was then reamplified in a secondary PCR reaction using a nested specific primer of the ACTH-R cDNA (oligonucleotide 2, Table 1) and the same anchor primer. Two separate PCR amplifications with the same oligonucleotides were done and all PCR experiments were performed in parallel with negative controls including the absence of cDNA template. Two other 3′-RACE were realized as above using oligonucleotides 3 and 4 and oligonucleotides 5 and 6 as primers specific of human ACTH-R cDNA. After sub-cloning, several clones corresponding to one given 3′-RACE product were dideoxysequenced. Shown in black bar is the coding region. The overlapping sequences of the cDNA clones obtained from 3′-RACE experiments are shown (PCR product A: bases 870–1423, PCR product B: bases 870–1599, PCR product C: bases 1440–2184, PCR product D: bases 1446–2810, PCR product E: bases 2508–3484). Black arrows indicate the positions of the polyadenylation signals.

Table 1
Sequences of the oligonucleotides

N	Sequences (5′ → 3′)	Position of the 1st base
1	ATCACCTTCACGTCGCTGTT	526
2	GATCTTCTGCAGCAGGTACT	870
3	AGGCCATTGCAGCCAATTTC	1322
4	TGTCTGTCTCCCACAGCCAT	1440
5	GCACATCCTGATTGTAAGTC	2228
6	TGGAGTCAAGTGAGGACAGT	2508

The oligonucleotides used were sense and numbered from their 5′-end to their 3′-end. Their positions are calculated from the ATG codon.

were identified (Figs. 2 and 3). One classical poly(A) signal at position 2163 for PCR product C and one variant poly(A) signal (ATTAAA) at position 2789 for PCR product D were observed; both associated with a poly(A) tail 21 bases downstream of the signal. In addition, a poly(A) signal at position 1837 was not followed by a poly(A) tail. A last 3′-RACE was done with oligonucleotides 5 and 6 and revealed a poly(A) signal at position 3465 with a poly(A) tail 19 bases downstream (PCR product E, Figs. 2 and 3). Since one major initiation site of transcription is located 177 bp away from the ATG codon [9] and assuming a poly(A) tail of 100–250 nucleotides [12,14,15], the lengths of these cDNAs were consistent with the transcripts of 1.8 kb (PCR products A and B), 3.4 kb (PCR product D) and 4 kb (PCR product E) seen on the Northern blot. Moreover, given the resolution of the Northern blot analysis, we cannot rule out the presence of an additional minor transcript explaining the PCR product C of 2184 bp. Sequence analysis of the 3′-flanking region of the human ACTH-R cDNA from the stop codon to the position 3484 then revealed seven potential poly(A) signals (Fig. 3). By 3′-RACE experiments, five different PCR products were amplified suggesting the presence of five functional poly(A) signals among the seven found in the 3′-UTR of the ACTH-R cDNA which has been analyzed. Four of them are classical (AATAAA) and one is aberrant (ATTAAA). Among the rare natural variants of the AATAAA poly(A) signal, ATTAAA is the most commonly found and mutational analysis shows that it is the mildest mutation in terms of its effect on poly(A) and cleavage efficiency [17]. The presence of several potential poly(A) signals in the 3′-non coding sequence has been reported in many other genes including human calmodulin I, rat luteinizing hormone receptor, human gonadotropin-releasing hormone receptor, human oestrogen receptor and human thyrotropin-releasing hormone receptor [18–22]. Among these poly(A) signals only a few were functional and the significance of the others remains unknown. In addition, sequence analysis of human ACTH-R cDNA revealed the presence of two copies of the ATTTA pentamer motif in the 3′-UTR that confer message instability [23,24]. Since one major initiation site of transcription has been reported for the human ACTH-R gene, the most likely molecular mechanism for the multiple ACTH-R mRNA transcripts is the alternative use of poly(A) signals. Such a mechanism has been described in other genes with multiple mRNA transcripts [18,19]. It is not clear what role alternative mRNA transcripts for a gene may play in gene regulation but it has been proposed that the presence of regulatory sequences in the 3′-UTR of mRNA may

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 892  TAGAATGGCTGATCCCTGGTTTTAGAATCCATGGGAATAACGTTGCCAAGTGCCAGAATA
 952  ATGTAACATTCCAACAAATGCCAGTGCTCCTCACTGGCCTTCCTTCCCTAATGGATGCAA
1012  GGATGATCCCACCAGCTAGTGTTTCTAATAGCTAGGTTCTATGTGAACAGTCTTATTGTA
1072  GGGGCAACCTCTTAACTTTGTGACTGGACAGATAAAACGATGTAGTAAAAGAAGGATAGA
1132  ATACAAAGTATTAGGTAGGTACAAAAGTAATTAAGGTTTTTGCCATTACTTTCAATGACC
1192  AAAAATTGCAATTACTTTTGCACCAATCTAGTAAAACAGCAATAAAAATTCAAGGGCTTT
1252  GGGCTAAGGCAAAGACTTGCTTTCCTGTGGACATCTAACAAGCCAGTTCTGAGGTGGCCT
1312  TTCCAGGTGGAGGCCATTGCAGCCAATTTCCAGAAGTTAAGTACCTGGACATGCGACTCC
1372  AGGCAGAAGATGTAGGGTCTCTGTAAGCCAATAATAAATTGGAAGGAATGCATTGCTGCA
1432  GCTGAATTTGTCTGTCTCCCACAGCCATGTGAATCTCCACCCTCCTCTTTCTCCCTGTTA
1492  GTCTGATGTATTGATGCCACCTCAGTTTCAGAAAGTAGGCTGAGTATAAACTATAAATGT
1552  CAAATAACGAGCTTCGAGTTTCCAATAATAAATGGACCTTCTCTGTTAGTCTTCTTTGCT
1612  CACTCAGTATCCCACTGGCCTTAAAACCCTTTCCTGTTACATTTCCTCATGCTTTATGAG
1672  CATACATTTCAAAGGAAGAAATGAAAATTTAATCCATTTAGTTCCCATGTGGAATACATA
1732  AAGGCCAGATGAAAATTGTCACTATTTGAAGAAGCTGTAACCAAACTATGTGTGTTTGTT
1792  ACAATGTAGAAGTACAAGAAAAGAGCCCCAACATGTATTTTAAGAAATAAAGAGAGAGAG
1852  ACAGAGACAGACAGAGAGAGAGAGAGAGAGAGAGAGAGAGTGTGTGTGTGTGTGTGTG
1912  TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATTTTCCCCATGCTTTTGGAC
1972  TATGGGGAAAACCAAAACCAAAGCAAGACTTCAAGCAATGGTGCTGTTATTATAGCCCCA
2032  AGTCAAAGACCTGAGGGAGGCAAACACCACCTCATTCTGCAGATGAATGTGAAAGCAGAC
2092  CCAGTCACTGGGAAATGTCATCCTCCCATCAGCCAAGATGCCAGCAATGGAAGAGTGGCA
2152  ACCCCAGTAGGAATAAAAGAAACATAATTTGCAAGTTCATTCATTTTTAATAGCTAAAAA
2212  TCAGCTTAAAGGAGAAGCACATCCTGATTGTAAGTCCCCACTAAGTTGGAGGGTGACTTG
2272  AATGGGGTGAAAGGTGAAAGGGACAGAGGAGAGCAGTGGGGCTTCAGAGGCCACCAGGCT
2332  CAGGATCTGCAGGATGGATGGTATCTTCCAGAACAGGCAATGCTTTGCCCTCAGGAGAAT
2392  TTCCCAGAGCTGCTGAGGGGAGAAGACAGCCACACACAGGACAGACCATTTGGTGATGGG
2452  TTTGATATTAGAAGTGGCAGGGACAGGAACTTCAGAAGCAAAGGAGGCAAGGCAGCTGGA
2512  GTCAAGTGAGGACAGTGGCAGGCGTGCTTTCACATGGCCTGTCCCACAGATGGAGGTGAA
2572  AGGTGCACCTTCTTGTCCTCTGTTCTGTAGAAATCCTTCCCTGTTTGATCCTTCCCCTGC
2632  CAAATGAACTATGTTACTCTAATACTAACCTGTATTAATTAATATATGAGATATATATAA
2692  GTTAATTTTTCATGAAATCTAAAGCACAACCCTAGAACTAATTTTTAAAAGTGTTATTTC
2752  TACCATTGAAAAAGTAATGTATAACATATTTTATGTGATTAAAGTGCGTATTCTCAATAA
2812  GAGGTAACCTTTTTTTGATGCTGCAATGCTCTGTGATACCACAGAGGTAAGCAATGCCAC
2872  TTAACCTGTATCATAAATAGTCCCAAACTGCTCTTCCTATAAAATTCTGCCTTTGTCAAC
2932  AGCTTTGCTGTCTCCTAATCACTCTCAAGCTCTCTGCTGTGCATGTGACTGTTGTCAGAA
2992  GGAAAATCACCAAGAAACTTCACCTCTCACTGCCTTTGATTTGTTGCAGTTAATCTAAGA
3052  AACAAAATGAAGATGGCTAGTCTAATGGTGGATGAAACAAAAATGAAGTCTGAGTGCTAA
3112  TTCAGAGAACTTGCAATTCCAGACATTTTCAATTCTAGGTCTTCTGCTATATTCCAATCA
3172  GAACAGAAGCTTCAGGGCTCATAGTTACTGAGAAAACTCACGTTTTTCTACCTCTAACTT
3232  CATATAGAATTCCAAATGAAAGCACCACCAAACTGCACATATTTGTGTGAGGAAGATCAA
3292  CAAGCTTCAGACTTTTCCCATGAGGACTTAATTCTTTTATCAAATTACCCAATTTTTAAA
3352  CTGCTGTGTGGATACTGTGAGTGTTCAGCTTTATCGATGACCTAGCCTTGGACCAGATAG
3412  CTGAAAATGTTCAGGATGTGTACTCAAGCTGATAGTAATTCTGAGCCCTGTCTAATAAAA
3472  AAGGAAGGATGTA
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Fig. 3. Sequence of the 3′-UTR of the human ACTH-R cDNA. Bases are numbered in the left margin, the position is calculated from the ATG codon. The 3′-UTR only is represented from the stop codon (TAG) to the position 3484. Polyadenylation signals are boxed and asterisks represent the sites of poly(A) addition. ATTTA pentamer motifs are underlined.

affect mRNA stability and translatability [25]. We speculate that sequences in the 3′-UTR may participate in the regulation of the ACTH-R gene expression.

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