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Separate Promoters in the Human A_1 Adenosine Receptor Gene Direct the Synthesis of Distinct Messenger RNAs that Regulate Receptor Abundance

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SUMMARY

There are two types of transcripts for the human A_1 adenosine receptor. They are expressed in a tissue-specific manner in human tissues and contain distinct exons. Previously, it had appeared that the two transcripts may have occurred through alternative splicing. The transcript β has two upstream AUG codons, which in transiently transfected COS-7 cells leads to a reduced level of receptor expression. When genomic sequence including sequences 5′ to transcriptional start site, exon 1A, intron 1A, exon 1B, intron 1B, exon 2, and coding sequence was inserted into an expression vector (pCMV5/huA1), the resulting transcripts had the same overall structure as the transcripts present in human tissues. Primer extension and 5′ rapid amplification of cDNA ends of mRNA from transfected cells revealed the transcription start sites for these two transcripts occurred in what previously had been termed introns. These results were confirmed with similar analysis of mRNA derived from human tissues. Two nonconsensus putative TATA boxes (TTAAGA and TTTAAA) are located upstream of the transcription start sites for transcripts α and β. When the TATA boxes and their flanking sequences were fused to a firefly luciferase gene containing promoterless vector, both demonstrated strong promoter activity in Chinese hamster ovary cells. Promoter A directs the synthesis of transcript α, and promoter B directs the synthesis of transcript β. Promoter A contains a series of AGG elements between the putative TATA box and the transcription start, which accounts for a major portion of the promoter activity based on deletion and mutation analysis. In general, promoter A is more active than promoter B in transfected cells. The nonconsensus TATA box in promoter B plays a more important role in promoter activity than the TATA box in promoter A. The human A_1 adenosine receptor gene appears to use two separate promoters to direct synthesis of distinct transcripts, which can then regulate the relative abundance of A_1 adenosine receptor in tissues. We have redefined the human A_1 adenosine receptor gene structure based on these new data.

Adenosine, an endogenous substance produced during cell metabolism, can induce various physiological responses, including protection of the heart from the injurious effects of ischemia and hypoxia (1). Many of the effects of adenosine and its analogues are produced via its receptors. At present, four adenosine receptors have been reported in the literature, A_1, A_{2a}, A_{2b}, and A_3 receptors (2). Since the cloning of these adenosine receptors, significant information about their structure, function, and regulation has emerged (3). We reported the genomic structure of the human A_1 adenosine receptor, the presence of two types of transcripts, and their tissue distributions (4). A partial genomic structure of the rabbit A_1 receptor gene has also been published (5). Based on the genomic and cDNA sequences isolated from human libraries, we previously reported that the human A_1 receptor gene contains at least six exons. Exons 1–4 are in the 5′-untranslated region. Exons 5 and 6 contain the coding sequence and the 3′-untranslated region. Two types of transcripts were found in a variety of human tissues containing either exons 3, 5, and 6 (Ex356) or exons 4–6 (Ex456). Ex456 is present in all tissues that express A_1 receptors, whereas Ex356 is present in selected tissues such as brain, testis, and kidney that express high levels of A_1 receptor (4). We recently described the importance of exon 4 in regulating A_1 receptor expression (6). There are two upstream ATG codons present in exon 4 but none in exon 3. When transfected into COS-7 cells, plasmids containing exon 4 expressed a much lower level of A_1 receptor than the plasmids without exon 4. After upstream ATG codons were mutated, the mutant plasmid expressed the same level of A_1 receptor as the plasmid containing Ex356. The upstream ATG codons in exon 4 may, therefore, be responsible for the constitutive low-level expression of A_1 receptor in some tissues. Moreover, during investigation of splicing patterns, we discovered the presence of two separate promoters for the human A_1 receptor and tested the promoter activity with a fused firefly luciferase

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ABBREVIATIONS: RACE, rapid amplification of cDNA ends; CHO, Chinese hamster ovary; PCR, polymerase chain reaction; bp, basepair(s).

gene transfected to CHO or COS-7 cells. Because extensive reverse transcription-PCR studies have failed to observe exons 1 and 2 in mature mRNA from human tissues and cells, we redefine the human A_1 adenosine receptor gene structure in the present report. What previously had been defined as exons 3, 4, 5, and 6 are now exons 1A, 1B, 2, and 3, respectively. Exons 1A and 1B have each increased in length at the 5′ ends as defined by the transcriptional start sites based on the two promoters found. Exon 1A has 267 bases, and exon 1B has 175 bases.

Both promoters have nonconsensus TATA boxes but no CAAT box. Promoter A produces a transcript that includes exons 1A, 2, and 3 that will be called transcript α. Promoter B produces a transcript that includes exons 1B, 2, and 3 that will be called transcript β.

Materials and Methods

Plasmid construction. The expression vector pCMV5 plus the human A_1 receptor coding sequence (4) was digested with appropriate restriction enzymes, and a fragment of genomic sequence in the 5′-untranslated region was inserted (Fig. 1, from +58 of promoter A), which contains part of exon 1A sequence; intron 1A, which includes promoter B, exon 1B and intron 1B; and exon 2 plus the receptor coding sequence of exon 3 in the expression vector and termed pCMV5/Gen5ut.

For promoter analysis, we inserted a genomic DNA fragment from human A_1 receptor gene into the promoterless vector pBLPniF with the firefly luciferase gene as a reporter gene (a gift from Dr. W. E. Kraus, Duke University Medical Center, Durham, North Carolina). The vector was digested with *Nhe*I and *Xho*I for cloning. The fragment containing promoter A (for pBLPniF/PmtA) begins from a natural *Nhe*I site at −897 relative to the transcriptional initiation site as +1 for exon 1A (Fig. 1) and ends at +106, which has an artificial *Xho*I site created by PCR. The insert containing promoter B (for pBLPniF/PmtB) extends from −422 (a natural *Nhe*I site in intron 1A) to +15 relative to the +1 site for exon 1B (Fig. 1). Both inserts were generated by PCR, and the template used was the genomic subclone B (4). The PCR fragments were digested with restriction enzymes (*Nhe*I and *Xho*I) and purified from agarose gel slices with the Qiaex DNA purification kit (Qiagen, Chatsworth, CA). The deleted inserts for both promoters A and B were produced either by PCR or with Erase-a-Base system (Promega, Madison, WI). All isolated clones were sequenced with Sequenase (United States Biochemical, Cleveland, OH) to ensure correct insert sequence and orientation or the end of deletion in the deletion mutants.

Mutants of AGG elements in promoter A, TATA box, and Ap1 binding site in promoter B. Because there are no convenient restriction enzyme sites around the desired mutations, the AGG elements in promoter A, TATA box, and Ap1 binding site in promoter B were mutated by blunt-end ligation of two PCR-produced mutant fragments (5′ and 3′ fragments). The templates used in PCR were pBLPniF/PmtA (for mutant AGG-1), AGG-1 (for mutant AGG-2), AGG-2 (for mutant AGG-3), and pBLPniF/PmtB (for mutant PMTBMUT). Vent DNA polymerase (New England Biolabs, Beverly, MA) was used in all PCR experiments, which, unlike Taq DNA polymerase, produces blunt-end fragments. The sense oligonucleotide for 5′ fragments (*niF-2*, 5′GTCCAAACTCATCAATGTATCTTATCAT3′) is part of the vector sequence close to the cloning site (*Nhe*I), and the antisense oligonucleotide for 3′ fragments (*Luc-2*, 5′AATCAGAGTGCTTTTGGCG-AAGAATGA3′) is in the middle of luciferase gene within the vector (Fig. 2). The antisense oligonucleotide for 5′ fragments (R indicates reverse) and the sense oligonucleotide for 3′ fragments (F indicates forward) split the targeted mutation region;

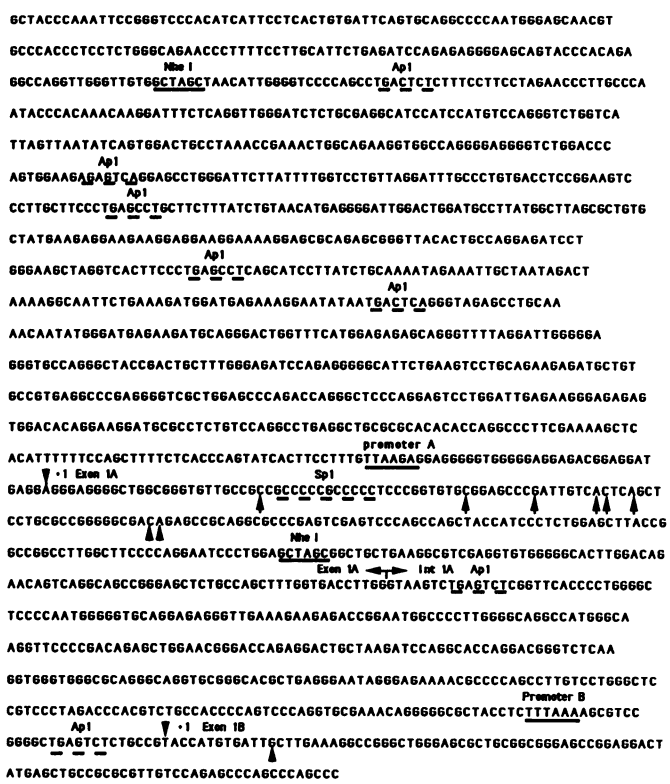

Fig. 1. Genomic DNA sequence of the human A1 adenosine receptor containing promoter A and promoter B. The putative nonconsensus TATA sequences for promoter A and B are underlined and labeled. Also underlined are *Nhe*I cloning sites for inserts in pBLPniF/PmtA and pBLPniF/PmtB, respectively. *Dashed lines*, sequences compatible with transcriptional factor (Ap1 and Sp1) binding sequences. *Arrows pointing downward*, transcriptional start sites. *Horizontal arrows*, border of intron/exon junctions. *Arrows pointing upward*, 5′ end of transcripts according to the results of 5′ RACE experiments.

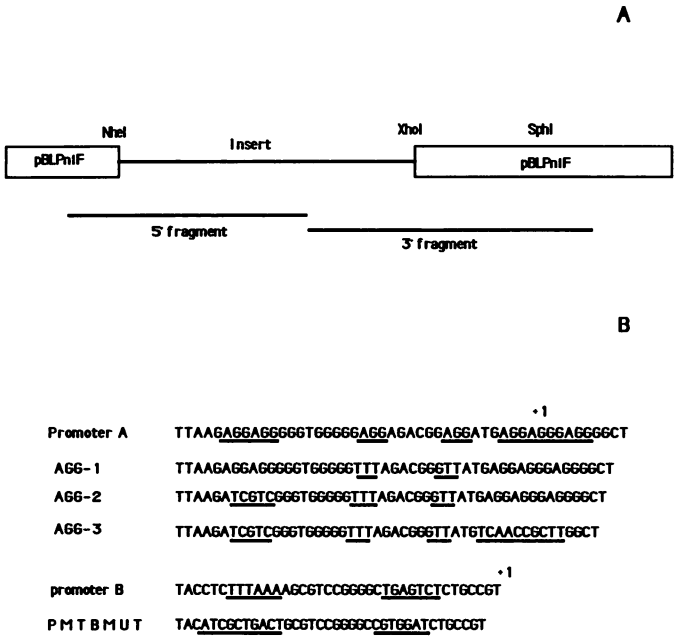

Fig. 2. Mutagenesis of AGG elements in promoter A and the TATA box and the Ap1 site in promoter B by PCR fragment ligation. A, Outline of mutant constructs; both 5′ and 3′ fragments were made by PCR with Vent DNA polymerase and specially designed oligonucleotides (see Materials and Methods). B, Wild-type sequences of promoters A and B and the mutants made from them. The target sequences and the mutated sequences are underlined.

they are AGG-1R, 5′TCTAAACCCCCACCCCCTCCTCTT3′; AGG-1F, 5′CGGG-TTATGAGGAGGGAGGGGCT; AGG-2R, 5′TCTTAACAAAGGAAGTGATACTGGG3′; AGG-2F, 5′TCGTCGGGTGGGGGTTTAGACGGGTT3′; AGG-3R, 5′CATAACCCGTC-TAAACCCCCA3′; AGG-3F, 5′TCAACCGCTTGGCTGGCGGGTGTTGCCGC3′; PMTBMUT, 5′ACGCAGTCAGCGATGTAGCGCCCCCTGTTTCGCA3′; and AP1MUT, 5′CCGGGGCCGTGGATCTGCCGTACCATGTGATTGCT3′. The 5′ fragments produced by PCR were digested with *Nhe*I, and the 3′ fragments were digested with *Sph*I (an unique site in the luciferase gene). The resulting fragments (5′ and 3′) were separated by agarose gel electrophoresis and purified with the Qiaex system. The two fragments were phosphorylated by T_4 DNA kinase (GIBCO-BRL, Gaithersburg, MD) and then ligated to each other and to the *Nhe*I/*Sph*I-digested vector by T_4 DNA ligase (GIBCO-BRL). The phosphorylation step can be omitted if the 5′ phosphorylated oligonucleotide is used in PCR. After the transformation of ligated plasmids in competent XL1-Blue (Stratagene, La Jolla, CA), the resulting colonies were sequenced to ensure that the appropriate sequence was obtained. Fig. 2 shows the mutated sequences for both promoters A and B. With this method of PCR fragment ligation, mutations can be made anywhere in the insert sequence regardless of whether any unique restriction enzyme site is present around the targeted location.

The plasmid DNAs were purified from 100 ml of LB/Amp culture via acidic phenol extraction (7).

Transfection of CHO or COS-7 cells. Cells were transiently transfected with the DEAE-Dextran transfection method in all experiments (8). COS-7 cells were grown in 25-cm^2 flasks (Corning) and CHO cells were grown in 35-mm plates (six-well plates) for transfection. CHO cells were cultured in F-12 nutrient mixture (HAM's F12) plus 10% fetal bovine serum, and COS-7 cells were grown in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum. In each transfection, 7 μg of the promoterless plasmid (pBLPniF) for the 25-cm^2 flask or 2.7 μg for the 35-mm plate, or the molar equivalent amount of the plasmids with insert, was used. For some promoter analysis experiments, CHO cells were cotransfected with a plasmid containing the β-galactosidase gene (*pSV-β-gal*). Each sample was transfected in triplicate, and each experiment was repeated at least three times.

Measurement of luciferase activity. At ~48 hr after transfection, the cultured cells were washed twice with phosphate-buffered saline and lysed in Promega's cell lysis reagent from the luciferase assay system for luciferase assays only and in reporter lysis buffer for assays of both luciferase and β-galactosidase. Luminescence was measured with a BioOrbit 1251 luminometer (Pharmacia LKB, Piscataway, NJ). β-Galactosidase activity was measured according to the standard method (8). For samples cotransfected with β-galactosidase, the results were normalized as luminescence/0.1 A_{420}. Data are presented as mean ± standard error values.

Primer extension. poly(A)$^+$ RNA (~3–5 μg) from the transfected cells or human tissues was used for each experiment. For promoter A, the primer used was Luc-1 (5′ATGTTTTTGGCGTCTTCCAT3′), and for promoter B, the primer was Ex1B (5′CAAAGTTCCAGGGAACA3′). The appropriate primers were first labeled with ^{32}P at the 5′ end using T_4 kinase (8). Reverse transcription was carried out with either the first-strand cDNA synthesis kit (Pharmacia) or the SuperScript reverse transcriptase (GIBCO-BRL) according to the manufacturer's instructions. The sample was run on a sequencing gel with a DNA sequencing sample using the same primer as standard.

5′ RACE/mRNA 5′ end mapping. Poly(A)$^+$ RNA from transfected cells or human tissues were used. The procedures of 5′ RACE essentially follow the protocol established by Frohman (9) with some modifications (10). Reverse transcription was performed with the first-strand cDNA synthesis kit (Pharmacia) with the NotId$(T)_{18}$ oligonucleotide as the primer. In general, two rounds of PCR were applied with the nested set of downstream primers located in A_1 receptor coding region or 5′-untranslated region. The upstream primers used in PCR were NotId$(T)_{18}$ and oligonucleotide NotI (5′-AACTGGAAGAATTCGCGGCCG-CAGGAA-3′). The second-round PCR products were cloned with the SureClone ligation kit (Pharmacia), and isolated clones were sequenced.

Results

We have previously reported evidence of the production of two distinct human A_1 receptor transcripts containing a single unique exon along with two common exons. The mRNAs appear to be expressed in a tissue-specific manner (4). The mechanism responsible for their production, whether by alternative promoters or splicing, was unclear. To probe potential mechanisms, we constructed the expression vector pCMV5/Gen5 ut (containing part of exon 1A plus I_{1A}, E_{1B}, I_{1B}, and E_2) and transfected it into COS-7 cells. Reverse transcription-PCR of total RNA purified from the transfected cells demonstrated two types of transcripts when primers corresponding to sequences in exon 1A and exon 1B were used, respectively. In addition, both transcripts were appropriately processed since the intervening introns were excised and no transcript with both exons 1A and 1B was observed. These are the same two transcripts found in human tissues (4).

Primer extension using an exon 1B sequence primer (Ex1B) showed that the 5′ end of the transcript was 21 bases upstream from what previously had been thought to be the 5′ border of the old exon 4 (Fig. 3A). Moreover, 5′ RACE experiments with poly(A)$^+$ RNA from human brain showed the 5′ end of the message to be in the old intron 3, albeit a few bases shorter than the transcript from transfected cells (Fig. 1). These results suggest that the transcript β is not a product of

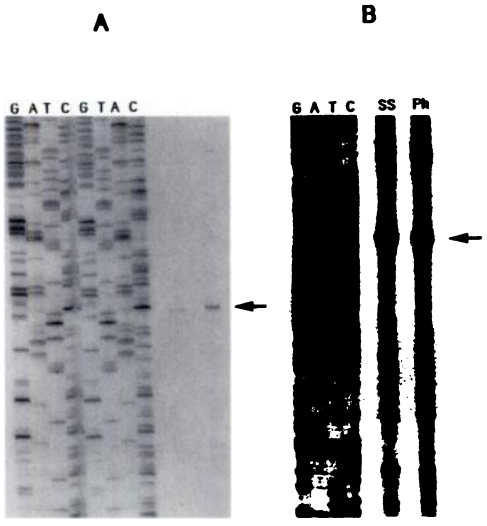

Fig. 3. Primer extension of poly(A)$^+$ RNA from transfected cells. Poly(A)$^+$ RNA was isolated from the transfected CHO or COS-7 cells (see Materials and Methods), and ^{32}P-labeled primers (Luc-1 or Ex1B) were used in reactions. *Arrows* point to the bands corresponding to the transcriptional start sites. A, The *sequencing sample on the left* side of the gel is used as standard for primer extension, which had pCMV5/Gen5 ut as template primed with the oligonucleotide Ex1B. The *two single lanes on the right* side of the gel are loaded with different amounts of primer extension reaction mix from the same sample, which is poly(A)$^+$ RNA from pCMV5/Gen5 ut-transfected COS-7 cells primed with Ex1B. B, The *sequencing sample on the left* is pBLPniF/PmtA as template primed with Luc-1. *Two single lanes on the right* are loaded with primer extension reaction mix from either SuperScript reverse transcriptase (*SS*) reaction or first-strand cDNA synthesis kit (Pharmacia) (*Ph*).

alternative splicing but rather a product of an independent promoter.

Primer extension of poly(A)$^+$ RNA extracted from the CHO cells transfected with pBLPniF/PmtA (promoter A expression plasmid) showed that the transcript from promoter A is initiated 167 bases upstream from the previously defined 5′ border of exon 3 (Fig. 3B). The 5′ RACE experiment with poly(A)$^+$ RNA from human brain tissue confirmed that the 5′ end of the transcript α is in the old intron 2 and that there are multiple 5′ ends scattered at 26–89 bases downstream from the +1 site identified by primer extension (Fig. 1). However, among the 20–30 sequenced 5′ RACE products from several experiments, there are no 5′ ends reaching the +1 site. This newly defined exon is called Ex1A.

Examination of the sequence upstream from the transcriptional start site (+1) in intron 1A reveals a TATA box-like sequence (TTTAAA) located 25 bases upstream from +1, and it is positioned at the appropriate location for a TATA sequence relative to transcriptional start sites (Fig. 1). This TATA-like sequence is surrounded by relatively GC-rich sequences, which is also common in promoter regions. When a 435-bp genomic sequence including this potential promoter region was fused with the promoterless firefly luciferase reporter gene (Fig. 4B) vector (pBLPniF/pmtB) and transfected into CHO cells, the expressed luciferase activity was ~23-fold higher than the promoterless vector (Fig. 4A).

When more than half of the insert sequence was deleted from the 5′ end (to −129), the promoter activity did not change significantly. Further deletion to a position close to the TATA box (to −39) significantly decreased the promoter activity by approximately one third. The deletion of TATA box (to −24) reduced by approximately two thirds the total promoter activity and one third of the original activity remains. This suggests that the short remaining sequence downstream of the TATA box contributes to the promoter activity. The above results confirmed the activity of this nonconsensus TATA box and its flanking sequence as the promoter (promoter B) for the transcript β (previously Ex456).

Because the 5′ end of exon 1A.2.3 is located further 5′ than previously thought, as determined by primer extension and 5′ RACE, we examined the sequence further upstream and found a putative nonconsensus TATA box (TTAAGA) 33 bp 5′ to the transcriptional start site. This nonconsensus TATA box has been reported to have promoter activity in human adenovirus E2-early gene (11). The transcriptional start site (+1) for this promoter (promoter A) was located by primer extension with poly(A)$^+$ RNA from cells transfected with the plasmid containing the structure shown in Fig. 5B. This promoter is flanked on the 5′ side of the putative TATA box by a relatively AT-rich sequence and on its 3′ side by an extended GC-rich sequence. The putative TATA box is located between −33 and −38 relative to the +1 site, a typical location for a consensus TATA box in vertebrate genes (Fig. 1). When the construct shown in Fig. 5B was tested for promoter activity, it showed almost 70-fold higher activity than the promoterless vector (Fig. 5A). Deletion of the upstream sequence to −253 somewhat reduced promoter activity. Further deletion to −89 increased the promoter activity to almost the original level, which may indicate the presence of a minor repressor element between −253 and −89. The sequence between −89 and −43 contributes some promoter activity, whereas deletion of the putative TATA box itself

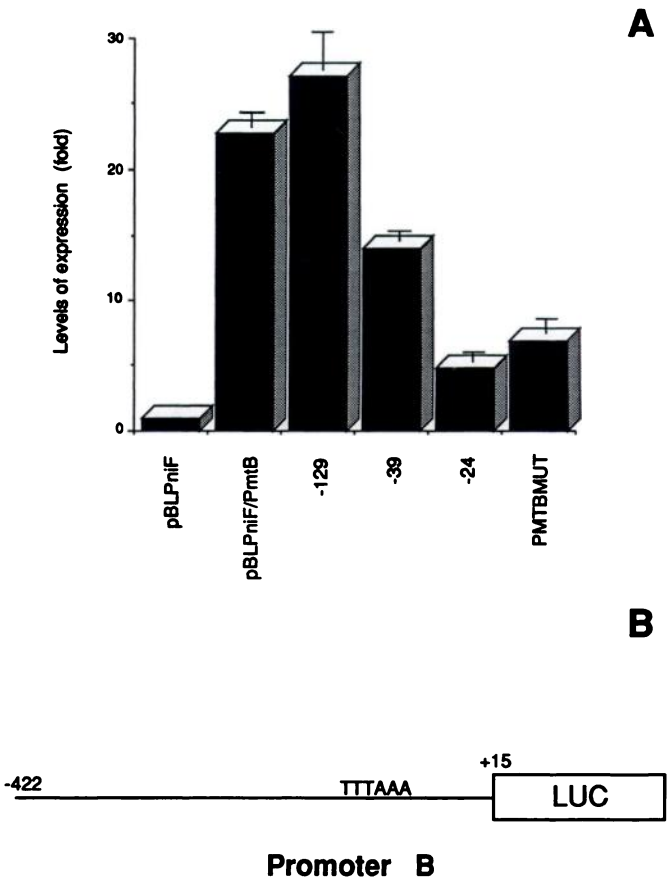

Fig. 4. Expression of promoter B in transiently transfected CHO cells. A, Luciferase activity is measured in cell extracts transfected with various lengths of A_1 receptor promoter sequence fused with firefly luciferase gene and the mutated promoter sequences. The 5′ end of pBLPniF/PmtB insert is located at −422 in relation to the transcriptional initiation site as +1. The level of expression is displayed as -fold compared with the luciferase activity of the no-promoter plasmid (three experiments). The position of deletion is indicated under each column. The luciferase activity has been normalized with the cotransfected β-galactosidase activity. B, Structure of the fused gene containing human A_1 receptor promoter B and its flanking sequences and the firefly luciferase gene. The TATA box is located between −26 and −31.

(TTAAGA) did not further reduce activity. A dramatic loss of promoter activity occurred after a 53-bp GC-rich sequence following the putative TATA box that includes the +1 transcriptional initiation site was deleted (Fig. 4A). The results show that the sequences surrounding the putative TATA box contribute the major promoter activity for the synthesis of transcript α. The TATA box is not likely acting as an essential promoter element.

The sequences between the TATA box and +1 site in promoter A contain multiple AGG elements, which have been shown to have positive regulatory activity in the rat thyroid hormone receptor α gene (12). Our deletion results also show substantial promoter activity in this region of the human A_1 receptor gene. This led us to test the effect of these AGG elements on the activity of promoter A. The mutations in this region created with PCR fragment ligation involve only the AGG elements (Fig. 2). The length of DNA insert was not changed, and the GC richness in this region was generally maintained. The results in Fig. 5A show that each additional mutation in the AGG elements reduces promoter activity progressively and that these AGG elements account for

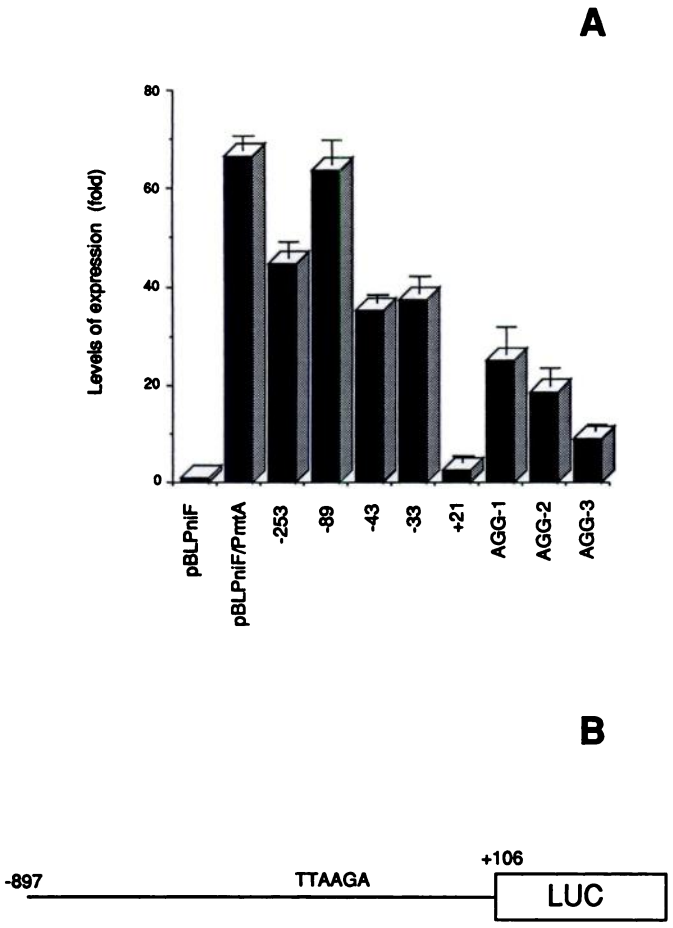

Fig. 5. Expression of promoter A in transiently transfected CHO cells. A, Luciferase activity is measured in cell extracts transfected with various lengths of A_1 receptor promoter sequence fused with firefly luciferase gene and the mutated promoter sequences. The 5′ end of pBLPniF/PmtA insert is located at −897 in relation to the transcriptional initiation site as +1. The level of expression is displayed as -fold compared with the luciferase activity of the no-promoter plasmid (three experiments). The position of deletion is indicated under each column. The luciferase activity has been normalized with the cotransfected β-galactosidase activity. B, Structure of the fused gene containing human A_1 receptor promoter A and its flanking sequences and the firefly luciferase gene. The putative TATA box is located between −33 and −39.

~85% of the total promoter activity in the DNA insert. Specifically, the mutant AGG-1 reduced promoter activity by ~60%, and the mutant AGG-2 gives a further reduction of 10%. The mutant AGG-3 has only ~15% of the original promoter activity, even though the remainder of the insert sequence is the same as the original (pBLPniF/PmtA). These results confirm the importance of the AGG elements in the promoter A activity.

For promoter B, the sequences between the TATA box and the transcriptional start contain an Ap1 binding site that may contribute promoter activity as defined by the deletion results above (Fig. 3A). A mutant with both the TATA box and the Ap1 binding site sequences mutated was created with PCR fragment ligation (Fig. 2). The results in Fig. 3A show that ~30% of the total promoter activity remains in this mutant, indicating the possible role of the sequence between −129 and −39 in promoter activity. By combining the results of deletional and mutational analysis, it can be deduced that the sequences between −129 and −39, the TATA box and the Ap1 binding site, can each account for approximately one third of the total promoter activity in promoter B. The presence of two AGG elements immediately upstream of the TATA box within the sequences between −129 and −39 is noteworthy, and they may be responsible for the promoter activity in this region.

Discussion

In the present report, we have shown that the human A_1 adenosine receptor gene has two independent promoters that initiate the synthesis of two distinct transcripts, transcript α (Ex1A.2.3) and transcript β (Ex1B.2.3). Promoter A initiates the synthesis of transcript α. During the splicing process, intron 1A, exon 1B, and intron 1B are removed as a unit (Fig. 6). Promoter B in intron 1A initiates the synthesis of transcript β and appears to operate at a low level in most human tissues. The two transcripts have different distributions among a variety of human tissues (4) and different translational properties (6). The two promoters regulating the synthesis of these transcripts are also different.

From the promoter activity analysis it is shown that promoter A has much higher activity than promoter B, at least in CHO cells (Figs. 3 and 4), and that much of its activity is related to the function of the AGG elements located between the probably inactive nonconsensus TATA box and the transcriptional start site. Both promoters A and B have nonconsensus TATA sequences. The importance of the TATA boxes versus their flanking sequences for the two promoters differs. The TATA sequence in promoter B appears to have a greater effect on promoter activity than the TATA box in promoter A. Although there are five potential Ap1 binding sites upstream of the nonactive TATA box in promoter A (Fig. 1), they do not have a significant effect on promoter activity in the cell line tested. Surprisingly, the bulk of promoter activity is related to the AGG elements rather than the TATA box. Although we have shown the importance of AGG elements for promoter activity, the real role they play in transcription initiation deserves further study. Similar AGG elements in the rat thyroid hormone receptor α gene have been shown to be a nuclear protein binding site (12). Perhaps the effect of AGG

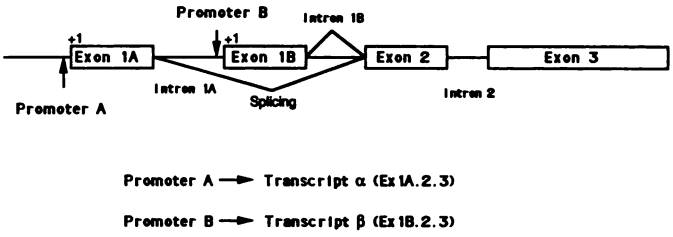

Fig. 6. Schematic location of human A_1 receptor gene promoters. Promoter A and promoter B locations are shown. Promoter A initiates the synthesis of the transcript α (Ex1A.2.3) from which intron 1A, exon 1B, and intron 1B are spliced out. This transcript is detected in some human tissues but not others. Promoter B initiates the synthesis of the transcript β (Ex1B.2.3), which is detected in all of the human tissues tested that express A_1 adenosine receptor.

elements on the promoter A activity is dependent on their position and the context of their surrounding sequences as there are many similar AGG elements upstream of the TATA box and their deletion had no effect on promoter activity. From the results observed with mutational analysis (Fig. 5A), it is clear that the greatest effect of the AGG elements on promoter A activity is a consequence of the AGGs mutated in the AGG-1 construct. Because the deletion of the sequence between −89 and −43 caused a >40% reduction in activity, this suggests that there may be additional regulatory sequences further upstream of the promoter A, and additional experiments are warranted. In the rat 5-hydroxytryptamine$_2$ receptor gene, the negative attenuating elements are located 2.5 kilobases 5′ to the transcriptional start site (13).

According to both deletion and mutation results (Fig. 3A), most of the promoter B activity appears to be related to the sequences between −129 and −39, the TATA box and the downstream Ap1 site.

The G protein-coupled receptors represent a very large gene family, and many receptors have been cloned. However, relatively little information is available regarding their gene structure and transcriptional regulation. Some receptor genes have been characterized as having TATA-less promoters, such as the mouse serotonin 2 (5-hydroxytryptamine$_2$) receptor (14), human D_{1A} dopamine receptor (15), and rat D_2 dopamine receptor (16). These promoter regions are GC rich and contain some transcriptional factor binding sites, such as Ap1, Ap2, and Sp1 sites. The human adrenergic receptors (α_{2A}, β_2, and β_3) have TATA-box containing promoters (17–19). Rat luteinizing-hormone receptor gene (20) and rat D_2 dopamine receptor gene (21) have two TATA-less promoters. Recently, three promoters for rat α_{1B}-adrenergic receptor gene (22) have been reported; two of them lack a TATA box, but one has both TATA and CAAT boxes. For the human A_1 adenosine receptor, we have characterized two independent nonconsensus TATA box-containing promoters (promoters A and B, although only one of the TATA boxes appears active) that initiate the synthesis of two different transcripts. One of the transcripts, β (Ex1B.2.3), has upstream ATG codons, which reduces the level of translational expression (6). At present, we have not located any of the known regulatory sequences, such as cAMP response elements or glucocorticoid response elements, within the inserts tested. Because we have documented that receptor expression is tissue specific, additional studies of the regulatory sequences responsible for their expression need to be undertaken.

The promoter analysis results we presented in this report reflect the complexity of gene regulation. Although we have characterized the minimal promoter regions for both transcripts (α and β), it remains possible that additional promoters are present.

Another unresolved question is how the human A_1 receptor gene expression is regulated by tissue- or cell-specific mechanisms or by any inducible factors. For the rat aromatic L-amino acid decarboxylase gene, two distinct promoters direct tissue-specific expression of two transcripts that have different 5′ untranslated sequences (23). In rat Fos-responsive gene *Fit-1*, two promoters generate mRNA isoforms with different 5′ and 3′ ends that code for either secreted or membrane-bound proteins related to type I interleukin-1 receptor (24). The use of the promoters thus determines the 3′ end splicing and the ratio of secreted to membrane-bound Fit-1 protein. For the human A_1 receptor, most tissues appear to express transcript β, but only select tissues express transcript α, and the mechanism of gene regulation involved needs further investigation.

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