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cDNA for the human β_2-adrenergic receptor: A protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor

(catecholamines/transmembrane signaling/opsins)

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ABSTRACT **We have isolated and sequenced a cDNA encoding the human β_2-adrenergic receptor. The deduced amino acid sequence (413 residues) is that of a protein containing seven clusters of hydrophobic amino acids suggestive of membrane-spanning domains. While the protein is 87% identical overall with the previously cloned hamster β_2-adrenergic receptor, the most highly conserved regions are the putative transmembrane helices (95% identical) and cytoplasmic loops (93% identical), suggesting that these regions of the molecule harbor important functional domains. Several of the transmembrane helices also share lesser degrees of identity with comparable regions of select members of the opsin family of visual pigments. We have localized the gene for the β_2-adrenergic receptor to q31-q32 on chromosome 5. This is the same position recently determined for the gene encoding the receptor for platelet-derived growth factor and is adjacent to that for the *FMS* protooncogene, which encodes the receptor for the macrophage colony-stimulating factor.**

Many hormones, neurotransmitters, and drugs influence cellular metabolic activities by stimulating the adenylate cyclase system, leading to the generation of the second messenger cAMP and activation of the cAMP-dependent protein kinase. The molecular components of this plasma membrane signaling system include specific receptors that bind ligands, the catalyst that converts ATP to cAMP, and guanine nucleotide regulatory or G proteins that functionally couple the receptors to the enzyme (1). The latter two components of the system have been purified and genes encoding several members of the "G protein family" have been cloned.

Of the receptors that are coupled to adenylate cyclase the only one that has been characterized in any detail is the β-adrenergic receptor (βAR). Two pharmacologically and physiologically distinct subtypes of this receptor, termed β_1AR and β_2AR, are both membrane glycoproteins of $M_r \approx$ 64,000 (2). Very recently, we reported cloning of cDNA and the gene for the hamster β_2AR (3). The deduced protein sequence indicated a protein of 418 amino acids, with seven clusters of hydrophobic amino acids likely representing membrane-spanning regions. This topology resembles that of the visual "light receptor" rhodopsin, which also possesses seven membrane-spanning domains (4–6).

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We now report the cloning and complete nucleotide sequence of the cDNA for the *human* β_2AR. While the receptor is highly similar to its hamster counterpart (87% of the amino acid residues are identical), significant regional differences in the extent of identity are noted.

METHODS

cDNA Library Screening. The human placenta cDNA library was kindly provided by Evan Sadler (Washington University School of Medicine). The cDNA was prepared from term placenta poly(A)$^+$ RNA and cloned in phage λgt11. The library contains 5×10^6 independent recombinants. The A431 λgt11 library was prepared from poly(A)$^+$ RNA from actively dividing A431 cells by methods previously described (3). It contains 1×10^6 independent recombinants. These libraries were probed with a ^{32}P-labeled 1.3-kilobase (kb) *Hin*dIII fragment from the hamster β_2AR genomic clone (3). Screening was performed according to the methods of Benton and Davis (7). A 2-kb cDNA clone, pTF (Fig. 1) obtained from the initial screen with the hamster probe was used to rescreen the placenta library. Low-stringency screening consisted of hybridizing and washing at 37°C in 6× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate). High-stringency screening was performed by hybridizing at 65°C in 6× SSC and washing at 65°C in 0.2× SSC.

DNA Sequencing. Sequencing of both strands of DNA was done by the dideoxy chain termination method (8, 9) from overlapping restriction fragments cloned in M13mp10 phage and pUC18, using the universal primer and oligonucleotide primers complementary to the cDNA.

Chromosomal Localization. Somatic cell hybrid analysis was carried out with 14 human–Chinese hamster hybrids of series XII, XIII, XVII, XVIII, and XXI and 2 human–rat hybrids of series XIX. The derivation and human chromosome content of these hybrids have recently been summarized (10). Southern blot analyses of *Eco*RI-digested genomic DNA from these hybrids and their parental controls were done with ^{32}P-labeled pTF as a probe.

In situ hybridization to human metaphase chromosomes was performed by using ^{3}H-labeled pTF as a probe according to methods previously described (10).

Abbreviations: βAR, β_1AR, and β_2AR, β-, β_1-, and β_2-adrenergic receptor; PDGF, platelet-derived growth factor; kb, kilobase(s); bp, base pair(s); GM-CSF, granulocyte-macrophage colony-stimulating factor; CSF-1, macrophage colony-stimulating factor.

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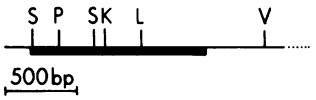

FIG. 1. Restriction map of the 2-kb cDNA clone pTF. S, *Sma* I; P, *Pst* I; K, *Kpn* I; L, *Bgl* II; V, *Pvu* II; bp, base pairs. The heavily shaded region represents the β_2AR coding sequence.

Blot Hybridization Analysis. DNA and RNA preparation, restriction enzyme analysis, and RNA and Southern blotting procedures were performed by methods described elsewhere (11). DNA probes were labeled by random primer extension (12).

RESULTS

We used a 1.3-kb *Hin*dIII fragment from the hamster β_2AR gene (3), which encodes virtually the entire receptor protein, to screen two human cDNA libraries. One cDNA library was constructed from term placenta poly(A)$^+$ mRNA in λgt11 and the other from human epidermoid carcinoma cell line A431 poly(A)$^+$ mRNA in λgt11. Term placenta specifically binds ≈200–250 fmol of ^{125}I-labeled cyanopindolol per mg of membrane protein, 40% with β_2AR and 60% with β_1AR specificity (ref. 13 and R.J.L. and M.G.C., unpublished data). A431 cells have ≈50,000 βARs per cell (14), all of the β_2 subtype. From 5×10^6 recombinants screened from the placenta library, 11 clones were obtained, while 2 clones out of 10^6 recombinants were obtained from the A431 library. Restriction mapping and Southern blot analysis of the placenta clones revealed 5 unique clones from the placenta library with inserts of 1.25–2 kb. The smaller clones had common restriction sites and hybridized at high stringency with oligonucleotides made complementary to various regions of the 2-kb clone. These smaller clones therefore represent fragments of the larger 2-kb clone. A restriction map of the 2-kb clone pTF is shown in Fig. 1 and the nucleotide sequence is shown in Fig. 2. A 2-kb clone from the A431 library (clone pHBHR3) has an identical restriction map.

The clones contain an open reading frame encoding a protein of 413 amino acid residues ($M_r \approx 46{,}000$) beginning with the methionine codon at position 1, which is flanked by nucleotides matching Kozak's criteria for efficient initiation of translation (15). The deduced amino acid sequence of the human β_2AR is that of a protein containing seven clusters of hydrophobic amino acids suggestive of membrane-spanning domains. Hydropathicity plots (not shown) of the human receptor are remarkably similar not only to those of the hamster receptor but to those of the family of visual pigments (4–6), the recently described human *mas* oncogene (16), and the *STE2* and *STE3* gene products of *Saccharomyces cerevisiae* (17). The latter are thought to represent receptors for the yeast α and **a** mating factors, respectively.

Comparison of the human and hamster (3) receptors is shown in Fig. 3. There is an overall amino acid residue identity of 87%, with the putative transmembrane helices showing 95% identity, the extracellular loops 75% identity, the cytoplasmic loops 93% identity, and the carboxyl terminus 74% identity. The carboxyl terminus is five amino acid residues shorter than that of the hamster β_2AR. The five-residue sequence found in the hamster β_2AR would be between residues 358 and 359 of the human receptor. Thus, the most divergent regions of the molecule are the amino and carboxyl termini. Other features that are conserved between the human and hamster receptors are two sites of N-linked glycosylation near the amino terminus, two consensus cAMP-dependent phosphorylation sites on presumed cytoplasmic domains, and a serine- and threonine-rich carboxyl terminus, possibly the locus of regulatory phosphorylation by the recently discovered enzyme βAR kinase (19).

```
                                                                    -111
TGGAACTGGCAGGCACCGCGAGCCCCTAGCACCGACAAGCTGAGTGTGCAGGACGAGTCCCCACCACACCCACACCAC
                                                                     -32
AGCCGCTGAATGAGGCTTCCAGGCGTCCGCTCGCGGCCCGCAGAGCCCCGCCGTGGGTCCGCCCGCTGAGGCGCCCCA
                                                                      36
                                  1
GCCAGTGCGCTTACCTGCCAGACTGCGCGCC ATG GGG CAA CCC GGG AAC GGC AGC GCC TTC TTG CTG
                                Met Gly Gln Pro Gly Asn Gly Ser Ala Phe Leu Leu
                                                                      96
GCA CCC AAT AGA AGC CAT GCG CCG GAC CAC GAC GTC ACG CAG CAA AGG GAC GAG GTG TGG
Ala Pro Asn Arg Ser His Ala Pro Asp His Asp Val Thr Gln Gln Arg Asp Glu Val Trp
          *                                          *
                                                                     156
GTG GTG GGC ATG GGC ATC GTC ATG TCT CTC ATC GTC CTG GCC ATC GTG TTT GGC AAT GTG
Val Val Gly Met Gly Ile Val Met Ser Leu Ile Val Leu Ala Ile Val Phe Gly Asn Val
                                                                     216
CTG GTC ATC ACA GCC ATT GCC AAG TTC GAG CGT CTG CAG ACG GTC ACC AAC TAC TTC ATC
Leu Val Ile Thr Ala Ile Ala Lys Phe Glu Arg Leu Gln Thr Val Thr Asn Tyr Phe Ile
                                                                     276
ACT TCA CTG GCC TGT GCT GAT CTG GTC ATG GGC CTG GCA GTG GTG CCC TTT GGG GCC GCC
Thr Ser Leu Ala Cys Ala Asp Leu Val Met Gly Leu Ala Val Val Pro Phe Gly Ala Ala
                                                                     336
CAT ATT CTT ATG AAA ATG TGG ACT TTT GGC AAC TTC TGG TGC GAG TTT TGG ACT TCC ATT
His Ile Leu Met Lys Met Trp Thr Phe Gly Asn Phe Trp Cys Glu Phe Trp Thr Ser Ile
                                                                     396
GAT GTG CTG TGC GTC ACG GCC AGC ATT GAG ACC CTG TGC GTG ATC GCA GTG GAT CGC TAC
Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu Cys Val Ile Ala Val Asp Arg Tyr
                                                                     456
TTT GCC ATT ACT TCA CCT TTC AAG TAC CAG AGC CTG CTG ACC AAG AAT AAG GCC CGG GTG
Phe Ala Ile Thr Ser Pro Phe Lys Tyr Gln Ser Leu Leu Thr Lys Asn Lys Ala Arg Val
                                                                     516
ATC ATT CTG ATG GTG TGG ATT GTG TCA GGC CTT ACC TCC TTC TTG CCC ATT CAG ATG CAC
Ile Ile Leu Met Val Trp Ile Val Ser Gly Leu Thr Ser Phe Leu Pro Ile Gln Met His
                                                                     576
TGG TAC CGG GCC ACC CAC CAG GAA GCC ATC AAC TGC TAT GCC AAT GAG ACC TGC TGT GAC
Trp Tyr Arg Ala Thr His Gln Glu Ala Ile Asn Cys Tyr Ala Asn Glu Thr Cys Cys Asp
                                                                     636
TTC TTC ACG AAC CAA GCC TAT GCC ATT GCC TCT TCC ATC GTG TCC TTC TAC GTT CCC CTG
Phe Phe Thr Asn Gln Ala Tyr Ala Ile Ala Ser Ser Ile Val Ser Phe Tyr Val Pro Leu
                                                                     696
GTG ATC ATG GTC TTC GTC TAC TCC AGG GTC TTT CAG GAG GCC AAA AGG CAG CTC CAG AAG
Val Ile Met Val Phe Val Tyr Ser Arg Val Phe Gln Glu Ala Lys Arg Gln Leu Gln Lys
                                                                     756
ATT GAC AAA TCT GAG GGC CGC TTC CAT GTC CAG AAC CTT AGC CAG GTG GAG CAG GAT GGG
Ile Asp Lys Ser Glu Gly Arg Phe His Val Gln Asn Leu Ser Gln Val Glu Gln Asp Gly
                                                                     816
CGG ACG GGG CAT GGA CTC CGC AGA TCT TCC AAG TTC TGC TTG AAG GAG CAC AAA GCC CTC
Arg Thr Gly His Gly Leu Arg Arg Ser|Ser Lys Phe Cys Leu Lys Glu His Lys Ala Leu
                                                                     876
AAG ACG TTA GGC ATC ATC ATG GGC ACT TTC ACC CTC TGC TGG CTG CCC TTC TTC ATC GTT
Lys Thr Leu Gly Ile Ile Met Gly Thr Phe Thr Leu Cys Trp Leu Pro Phe Phe Ile Val
                                                                     936
AAC ATT GTG CAT GTG ATC CAG GAT AAC CTC ATC CGT AAG GAA GTT TAC ATC CTC CTA AAT
Asn Ile Val His Val Ile Gln Asp Asn Leu Ile Arg Lys Glu Val Tyr Ile Leu Leu Asn
                                                                     996
TGG ATA GGC TAT GTC AAT TCT GGT TTC AAT CCC CTT ATC TAC TGC CGG AGC CCA GAT TTC
Trp Ile Gly Tyr Val Asn Ser Gly Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe
                                                                    1056
AGG ATT GCC TTC CAG GAG CTT CTG TGC CTG CGC AGG TCT TCT TTG AAG GCC TAT GGG AAT
Arg Ile Ala Phe Gln Glu Leu Leu Cys Leu Arg Arg Ser|Ser Leu Lys Ala Tyr Gly Asn
                                                                    1116
GGC TAC TCC AGC AAC GGC AAC ACA GGG GAG CAG AGT GGA TAT CAC GTG GAA CAG GAG AAA
Gly Tyr Ser Ser Asn Gly Asn Thr Gly Glu Gln Ser Gly Tyr His Val Glu Gln Glu Lys
                                                                    1176
GAA AAT AAA CTG CTG TGT GAA GAC CTC CCA GGC ACG GAA GAC TTT GTG GGC CAT CAA GGT
Glu Asn Lys Leu Leu Cys Glu Asp Leu Pro Gly Thr Glu Asp Phe Val Gly His Gln Gly
                                                                    1236
ACT GTG CCT AGC GAT AAC ATT GAT TCA CAA GGG AGG AAT TGT AGT ACA AAT GAC TCA CTG
Thr Val Pro Ser Asp Asn Ile Asp Ser Gln Gly Arg Asn Cys Ser Thr Asn Asp Ser Leu
                                                                    1313
CTG TAA AGCAGTTTTTCTACTTTTAAAGACCCCCCCCCCCCCAACAGAACACTAAACAGACTATTTAACTTGAGGGT
Leu ---
                                                                    1392
AATAAACTTAGAATAAAATTGTAAAAATTGTATAGAGATATGCAGAAGGAAGGGCATCCTTCTGCCTTTTTTATTTTTT
                                                                    1471
TAAGCTGTAAAAAGAGAGAAAACTTATTTGAGTGATTATTTGTTATTTGTACAGTTCAGTTCCTCTTTGCATGGAATTT
                                                                    1550
GTAAGTTTATGTCTAAAGAGCTTTAGTCCTAGAGGACCTGAGTCTGCTATATTTTCATGACTTTTCCATGTATCTACCT
                                                                    1629
CACTATTCAAGTATTAGGGGTAATATATTGCTGCTGGTAATTTGTATCTGAAGGAGATTTTCCTTCCTACACCCTTGGA
                                                                    1708
CTTGAGGATTTTGAGTATCTCGGACCTTTCAGCTGTGAACATGGACTCTTCCCCCACTCCTCTTATTTGCTCACACGGG
                                                                    1787
GTATTTTAGGCAGGGATTTGAGGAGCAGCTTCAGTTGTTTTCCCGAGCAAAGGTCTAAAGTTTACAGTAAATAAAATGT
                  1810
TTGACCATGAAAAAAAAAAAAA
```

FIG. 2. Nucleotide and deduced amino acid sequence of the human β_2AR cDNA clone pTF. The ATG and the termination codon, TGA, in the 5′ untranslated region are underlined. Within the coding sequence the sites of N-linked glycosylation are indicated by *. The consensus cAMP-dependent protein kinase A phosphorylation sites are boxed. In the 3′ untranslated region three potential polyadenylylation sequences are underlined.

Comparison of the human β_2AR with the other integral membrane proteins mentioned above reveals that several of the putative transmembrane helices share homology with comparable regions of select members of the visual pigment family (4–6). For example, hydrophobic domain 1 of the human β_2AR shares 36% identity with the comparable region of human red and green opsin. Hydrophobic domain 2 of the human β_2AR shares 30% identity with the comparable region of bovine rhodopsin and 34% identity with the comparable region of human rhodopsin. In this region the sequence Leu-Ala-Xaa-Ala-Asp-Leu is seen in all of the visual pigments except human blue opsin. Hydrophobic domain 5 of the human β_2AR shares 25% identity with the comparable regions of human and bovine rhodopsin and 28% identity with

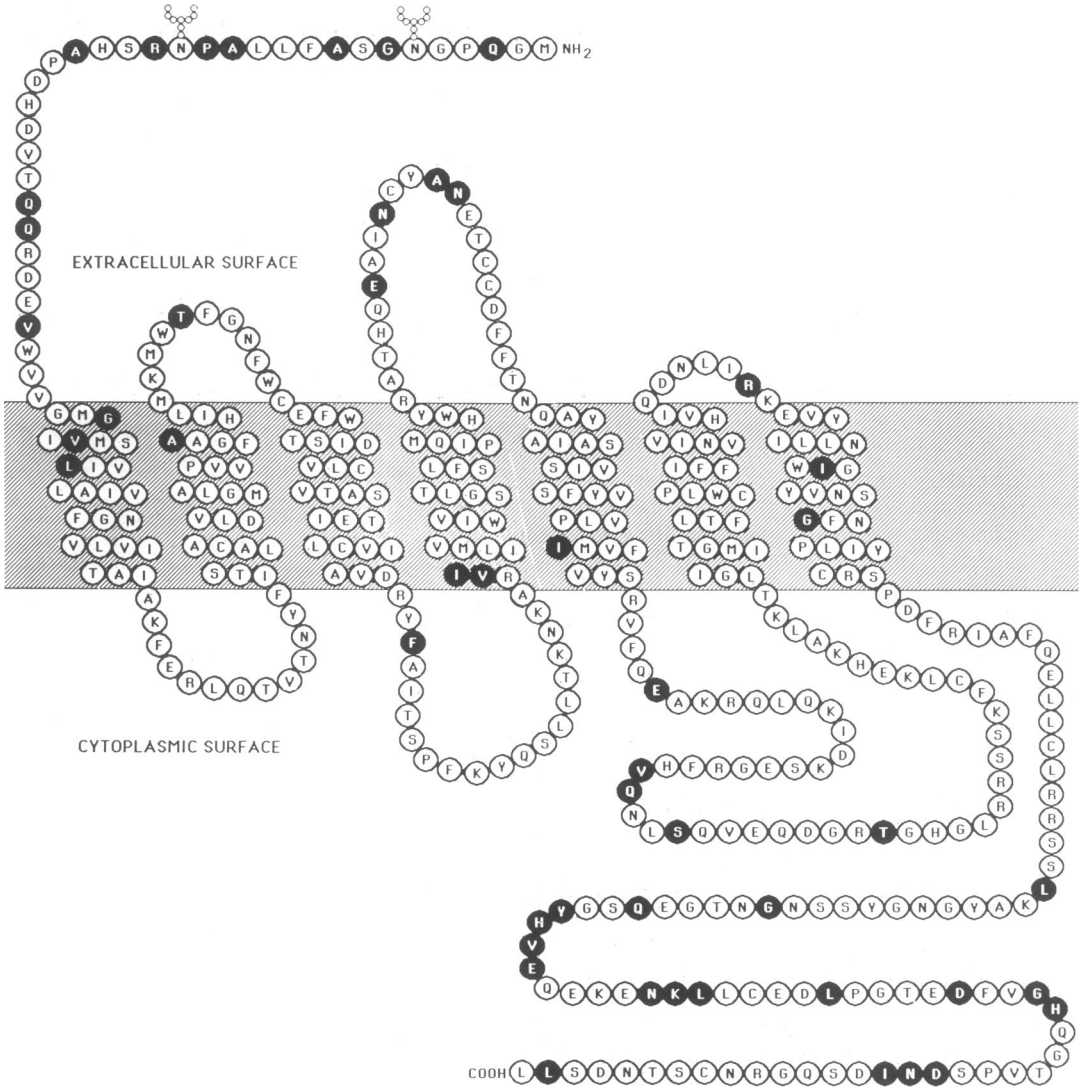

FIG. 3. Proposed model for insertion of the β_2AR in the cell membrane. The model is based on hydropathicity analysis of the human β_2AR according to the methods of Kyte and Doolittle (18). The standard one-letter code for amino acid residues is used. Hydrophobic domains are represented as transmembrane helices. Black circles with white letters indicate residues in the human sequence that differ from those in hamster. Also noted are the potential sites of N-linked glycosylation.

the comparable region of human blue opsin. Hydrophobic domain 6 of the human β_2AR shares 29% identity with regions 1 and 6 of bovine rhodopsin.

Blot hybridization analysis of poly(A)$^+$ mRNA from term human placenta and A431 cells is shown in Fig. 4. Only one band, of approximately 2.2 kb, was observed when nick-translated clone pTF was used as a probe. The size of this transcript is only slightly larger than that of the largest clones obtained from the two cDNA libraries, suggesting that these clones represent almost full-length copies of this mRNA species. This blot also illustrates the rarity of β_2AR mRNA. The signal produced from probing 20 μg of poly(A)$^+$ RNA from term placenta with ^{32}P-labeled actin cDNA is much stronger than that obtained by probing 90 μg of the same RNA preparation with ^{32}P-labeled pTF of an equivalent specific activity.

The 5′ untranslated region in both human and hamster cDNAs has an AUG triplet upstream of the initiator methionine codon. The location is 101 bp upstream for the human and 111 bp upstream for the hamster (3). In both the human and hamster transcripts the AUG is followed by a 19-codon open reading frame. There is 85% nucleotide sequence identity over this region and 12 of the 19 amino acids in the short open reading frame are identical. The nucleotides surrounding these AUG triplets do not fit Kozak's consensus (15) for an efficient initiator of translation.

As noted above, human placenta contains both β_1- and β_2AR subtypes, yet the coding sequences of all cDNA clones obtained from this library are identical to those obtained from the A431 (100% β_2 subtype) library on the basis of restriction mapping and partial DNA sequencing of the A431 clone. This, together with the extensive homology observed between our human clones and the hamster β_2AR, suggests that we have cloned the human β_2AR. The placenta library was also screened at low stringency, but no additional clones were obtained. Given the structural similarities observed between the β_2AR and the β_1AR by peptide mapping (20) and antibody cross-reactivity (3, 21), we would expect the β_1AR cDNA to be homologous with the β_2AR cDNA. Our inability to obtain a β_1-specific clone by low-stringency screening of the placenta library (60% β_1-specific binding in placenta tissue) might be explained in several ways: (*i*) the nucleotide sequences of β_1- and β_2AR cDNAs are more divergent than biochemical and immunologic comparison of these two proteins might suggest; (*ii*) the abundance of β_1AR-specific mRNA in placenta tissue is much lower than that for β_2AR-specific mRNA; (*iii*) β_1- and β_2ARs are coded for by

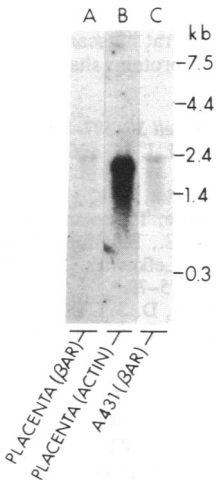

FIG. 4. Blot hybridization analyses of poly(A)$^+$ RNA from human placenta tissue (90 μg) and A431 cells (37 μg) probed with ^{32}P-labeled pTF and from human placenta tissue (20 μg) probed with ^{32}P-labeled actin cDNA. Hybridization was performed in 6× SSC at 65°C. Filters were washed in 0.2× SSC at 65°C. Autoradiograms were developed after 24-hr exposure. Positions of molecular weight standards are indicated at right.

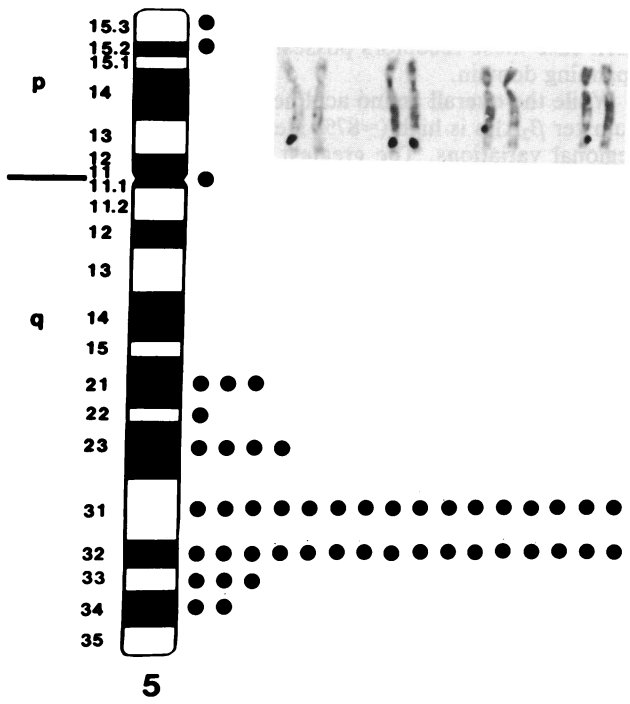

FIG. 5. Diagram of chromosome 5, indicating autoradiographic silver grain distribution resulting from *in situ* hybridization with ^{3}H-labeled pTF. Each dot represents one grain. (*Inset*) Representative pairs of chromosome 5 with typical labeling.

the same message but the translation products are processed differently.

Chromosomal Location. Chromosomal localization was accomplished by somatic cell hybrid analysis and *in situ* hybridization to metaphase chromosomes. Data obtained by Southern blot analysis of *Eco*RI-digested genomic DNA from somatic cell hybrids are summarized in Table 1. Hybridization to the human-specific *Eco*RI fragment is observed only in hybrids containing human chromosome 5. Every other chromosome can be excluded by three or more discordant hybrids.

This chromosomal location was independently confirmed by *in situ* hybridization of ^{3}H-labeled pTF to human metaphase chromosomes. Thirty out of 100 cells analyzed exhibited silver grains on bands q31-q32 of one or both chromosomes 5 (Fig. 5). Of 48 grains observed on chromosome 5, 32 were located at q31-q32. Furthermore, grains over this specific region represented 17.4% (32/184) of all chromosomal label and no other site was labeled above background. Thus, the gene for the β_2AR is regionally localized to chromosome 5, bands q31-q32.

DISCUSSION

Seven Membrane-Spanning Domains—A Feature of Membrane Receptors Coupled to G Proteins? Like its hamster counterpart, and the opsin family of visual pigments, the human β_2AR appears to contain seven membrane-spanning domains. Several other membrane proteins have recently been shown to have amino acid sequences compatible with seven membrane-spanning domains. These include the α and **a** mating factor receptors of yeast (*STE2* and *STE3* gene products) (17) and the recently described *mas* oncogene (16).

Both the βAR and rhodopsin are coupled to members of the G protein family of signal-transducing proteins, G_s and transducin, respectively. The α mating factor receptor of yeast has been reported to inhibit adenylate cyclase (17). It is possible, though currently unknown, that both the *STE3* and *mas* gene products might also be coupled to G proteins. An interesting speculation is that the seven membrane-spanning region feature might be common to all the many membrane receptors that are coupled to G proteins. It should also be noted in this regard that the structure of the βAR is quite different from that of several other membrane receptors whose structures are currently known. These include the receptors for low density lipoprotein (22), transferrin (23),

Table 1. Correlation of human pTF sequences with human chromosomes in rodent–human somatic cell hybrids

Hybridization/ chromosome*	Human chromosome																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
+/+	1	1	3	3	4	4	1	2	2	0	4	2	3	3	2	2	2	3	3	3	2	3	1	0
−/−	11	11	3	8	11	4	6	4	8	10	7	5	4	3	4	5	11	7	9	8	3	6	5	10
+/−	3	2	1	1	0	0	3	2	2	4	0	2	1	1	2	2	2	1	1	1	1	1	1	4
−/+	1	1	7	3	0	7	3	6	3	1	5	5	8	7	8	5	1	4	3	3	8	5	2	2
Discordant hybrids	4	3	8	4	0	7	6	8	5	5	5	7	9	8	10	7	3	5	4	4	9	6	3	6
Informative hybrids	16	15	14	15	15	15	13	14	15	15	16	14	16	14	16	14	16	15	16	15	14	15	9	16
Percent discordant	25	20	57	27	0	47	46	57	33	33	31	50	56	57	63	50	19	33	25	27	64	40	33	38

Data are derived from Southern blot analyses of genomic DNA of hybrid cells. Data on rearranged chromosomes or chromosomes present in fewer than 10% of the hybrids were excluded.

*Hybridization/chromosome indicates hybridization to human β_2AR *Eco*RI fragment observed/somatic cell hybrid contains indicated chromosome.

insulin (24), epidermal growth factor (25), and PDGF (26). In each case these receptors possess only a single membrane-spanning domain.

While the overall amino acid homology of the human and hamster β_2ARs is high (≈87% identity) there are significant regional variations. The greatest similarity is found in the putative transmembrane helices and cytoplasmic loops, where 95% and 93%, respectively, of the amino acid residues are identical. The overall similarity between the human β_2AR and the visual pigments is low; however, as noted in *Results*, several of the hydrophobic domains share 25–36% identity with the comparable regions of select members of the family of visual pigments. Given the tendency toward conservation of structure in functionally important regions of proteins, these observations may further underscore the functional importance of the membrane-spanning regions.

As with the hamster receptor, the human receptor retains a very serine- and threonine-rich carboxyl terminus, which may serve as the locus of regulatory phosphorylation by the recently discovered βAR kinase (19). This enzyme appears to function as does rhodopsin kinase, phosphorylating the agonist-occupied receptor and uncoupling it from G protein interaction, thus leading to "desensitization."

5′ Untranslated Region. As noted in the results, the 5′ untranslated regions in both human and hamster cDNAs have AUG triplets upstream of the initiator methionine codon for the β_2AR. This feature is observed in approximately 5% of eukaryotic messages (15) and is also present in the mRNA for the estrogen receptor (27). The high degree of homology seen between the human and hamster transcripts in this region suggests that these segments may have functional significance. There is some evidence from the study of the yeast *GCN4* gene that such upstream AUG triplets may be important in regulating translation (28).

Chromosomal Location. The gene for the β_2AR had been provisionally assigned to chromosome 5 by expression studies in somatic cell hybrids (29). Our assignment of the gene for the β_2AR to 5q31-q32 is consistent with these results. At the cytological level, the map position of the gene for the β_2AR is exactly the same as that for the receptor for PDGF (26). Three other cloned genes have been mapped to the distal long arm of chromosome 5. The gene encoding granulocyte-macrophage colony-stimulating factor (GM-CSF) is at 5q23-q31, probably proximal to the genes for the β_2AR and the PDGF receptor (30). The protooncogene *FMS*, a gene whose product is related to or identical to the receptor for macrophage colony-stimulating factor (CSF-1), is located at 5q33 or q34, distal to the genes for the β_2AR and the PDGF receptor (30), and CSF-1 is at 5q33.1 (31). None of these proteins has sequence homology with the βAR.

A distinct constellation of cytogenetic and clinical features is associated with deletions of 5q (bands q13-q33) including the region where we have localized the gene for the βAR, and these have been reviewed by LeBeau *et al.* (30). Such patients have refractory anemia, morphologically abnormal megakaryocytes in the bone marrow, and a tendency toward thrombocytosis. GM-CSF, CSF-1, and FMS are involved in hematopoietic maturation and may play a role in the development of the hematological disorders associated with these partial 5q deletions. The β_2AR has no sequence homology with the PDGF receptor, GM-CSF, or FMS and is not known to be functionally or evolutionarily related to these proteins. Moreover, the biological significance, if any, of the physical proximity of these various loci on human chromosome 5 also remains to be clarified. Cells from patients with various forms of 5q syndrome should provide useful materials for approaching such questions as well as for exploring the effects of such deletions on βAR expression.

Note Added in Proof. After this paper was communicated, clonings of the avian βAR (32) and rat muscarinic cholinergic receptor (33) revealed that both of these proteins share homology with the hamster β_2AR (3) and rhodopsin.

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