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Chromosomal organization of adrenergic receptor genes

(gene localization/pulsed field gel electrophoresis/comparative mapping)

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ABSTRACT **The adrenergic receptors (ARs) (subtypes α_1, α_2, β_1, and β_2) are a prototypic family of guanine nucleotide binding regulatory protein-coupled receptors that mediate the physiological effects of the hormone epinephrine and the neurotransmitter norepinephrine. We have previously assigned the genes for β_2- and α_2-AR to human chromosomes 5 and 10, respectively. By Southern analysis of somatic cell hybrids and *in situ* chromosomal hybridization, we have now mapped the α_1-AR gene to chromosome 5q32→q34, the same position as β_2-AR, and the β_1-AR gene to chromosome 10q24→q26, the region where α_2-AR is located. In mouse, both α_2- and β_1-AR genes were assigned to chromosome 19, and the α_1-AR locus was localized to chromosome 11. Pulsed field gel electrophoresis has shown that the α_1- and β_2-AR genes in humans are within 300 kilobases (kb) and the distance between the α_2- and β_1-AR genes is <225 kb. The proximity of these two pairs of AR genes and the sequence similarity that exists among all the ARs strongly suggest that they are evolutionarily related. Moreover, they likely arose from a common ancestral receptor gene and subsequently diverged through gene duplication and chromosomal duplication to perform their distinctive roles in mediating the physiological effects of catecholamines. The AR genes thus provide a paradigm for understanding the evolution of such structurally conserved yet functionally divergent families of receptor molecules.**

The adrenergic receptors (ARs) are plasma membrane receptors mediating the physiological effects of neurotransmitters, hormones, and drugs. ARs belong to the family of receptors that are coupled to guanine nucleotide binding regulatory proteins (G proteins) (1, 2). This receptor family also includes rhodopsin, the visual color opsins, muscarinic cholinergic receptors, and many other neurotransmitter receptors and receptors for peptide hormones. There are two major classes of AR, α and β, each of which has pharmacologically distinguishable subtypes (α_1 and α_2, β_1 and β_2). The β_1- and β_2-ARs activate adenylate cyclase, while the α_2-AR inhibits that enzyme. The α_1-AR stimulates phospholipase C.

The most striking structural feature of the ARs and other G protein-coupled receptors is the presence of seven stretches of hydrophobic amino acids, which are believed to form seven α-helices that span the lipid bilayer of the cell membrane. Amino acid sequence homology among ARs is highest in these transmembrane domains (3, 4). This feature is shared with other G protein-coupled plasma membrane receptors (5–7).

We have previously assigned the genes encoding platelet α_2- and β_2-ARs to human chromosomes 10q24→q26 and 5q31→q33, respectively (8, 9). Two α_2-AR-related sequences were localized to human chromosomes 2 and 4 (8). The β_2-AR gene was mapped to chromosome 18 in the mouse (10). Here we report the chromosomal assignment of β_1- and α_1-AR genes in human and the assignment of the three remaining AR genes in mouse. Interestingly, we have found that the α_1-AR gene localized to human chromosome 5q32–q34, in the close vicinity of the β_2-AR locus, and the β_1-AR gene is mapped to human chromosome 10q24–q26, the same region as the platelet type α_2-AR gene. In an attempt to determine the physical distances between α_1- and β_2-AR loci, and β_1- and α_2-AR loci, pulsed field gel electrophoresis was performed. The results suggest that the evolutionary mechanism for the AR gene family is gene duplication followed by chromosomal duplication.

MATERIALS AND METHODS

Hybridization Probes. A 1.6-kilobase (kb) *Sma* I/*Hin*dIII genomic fragment composed of 1.2 kb of coding sequence and 0.4 kb of intervening sequence of hamster α_1-AR was used to localize the α_1-AR gene in human and mouse (11). The mouse β_2-AR locus was mapped by hybridization to a 2-kb *Bam*HI/*Pst* I fragment from the 5′ untranslated region of the human platelet α_2-AR gene (8). This probe only recognizes the α_2-AR sequence on human chromosome 10. A 1.3-kb *Sma* I cDNA fragment of human β_1-AR was used to determine the β_1-AR gene location in human and mouse (12). The β_2-AR probe pHBHR3 is a 2-kb human cDNA fragment representing the entire coding region plus a 14-base-pair poly(A) tail of the β_2-AR gene (9). All the probes described above were also used for hybridizing filters generated by pulsed field gel electrophoresis.

Hybrid Cell Lines. Panels of selected Chinese hamster–human hybrid cell lines of series XII, XIII, XV, XVII, XVIII, XXI, and 31 were used to assign α_1- and β_1-AR genes to human chromosomes. Derivation of the hybrid cell lines has been recently summarized (13). Localization of the AR genes in mouse was accomplished by analyzing panels of mouse–Chinese hamster hybrid cell lines of series I, EAS and EBS, and of a mouse–rat hybrid RTM9. The hybrids were characterized chromosomally and biochemically as described (13). When hybrid cell lines were expanded in cell culture for DNA extraction, their chromosome constitution was reexamined by trypsin Giemsa banding.

Southern Blot Hybridization. Genomic DNA was extracted from hybrids and parental control cell lines according to standard methods. DNA samples were digested with restriction endonuclease, separated by electrophoresis on agarose gels, transferred to Hybond nylon (Amersham) filters, and hybridized with ^{32}P-labeled probes as described (13, 14).

***In Situ* Hybridization.** DNA probes were nick-translated with [^{3}H]dATP, [^{3}H]dCTP, and [^{3}H]dTTP to a specific activity of 2–3 × 10^7 cpm/μg. Hybridization to human chromosome preparations, posthybridization wash, emulsion au-

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Abbreviation: AR, adrenergic receptor.

toradiography, and silver grain analysis were carried out according to the published procedures (13).

Pulsed Field Gel Electrophoresis. Lymphoblastoid cells and fibroblasts from six individuals were used for this study. The methods for genomic DNA preparation in agarose blocks, the preparation of λ multimers and yeast chromosomes as DNA size markers, the restriction digestion of DNA in agarose blocks, and the condition of pulsed field gel electrophoresis have been described (15). A contour-clamped homogeneous electric field apparatus (Chef II; Bio-Rad) has been used in the analysis (16). The electrophoresis was conducted with 1% agarose gel at 150–200 V in 0.5× TBE (1× TBE = 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3) for 30–50 hr at 15°C with continuously increasing pulse times of 50–90 or 120–180 sec. After electrophoresis, the DNA was transferred to Hybond nylon filters as in regular Southern blotting and sequentially hybridized with probes of the AR genes. Prehybridization and hybridization were done as described (13, 14). Filters were stripped with 0.1 M NaOH/1% SDS and exposed to autoradiographic films between hybridizations to ensure that the previous signals were cleaned out.

RESULTS

α_1- and β_1-AR Mapping in the Human. Southern blot analysis of DNA from 13 human–Chinese hamster hybrid cell lines assigned the α_1-AR gene to human chromosome 5 (Table 1). The human-specific 7-kb *Bgl* II, 3.3-kb *Pst* I, and 1.7-kb *Eco*RI fragments were present in hybrids containing human chromosome 5, but not in hybrids lacking chromosome 5.

Assignment of the β_1-AR locus to human chromosome 10 was made by Southern analysis of 12 human–Chinese hamster hybrids. The 6.3-kb *Hin*dIII, 8.5-kb *Bgl* II, and 13.5-kb *Eco*RI human-specific bands segregated with human chromosome 10 (Table 1). In addition, the 8.4-kb *Eco*RI, 15-kb *Bgl* II, and 9.0-kb *Hin*dIII bands were present in human control DNA but were not concordant with a single chromosome in this hybrid panel. Both *Eco*RI fragments were observed in DNA from 14 unrelated individuals; therefore, the 8.4-kb band does not likely represent a polymorphism. *In situ* hybridization did not reveal a secondary site. Cross-hybridization fragments, which are 18.5-kb *Eco*RI, 5.5-kb *Bgl* II, and 5-kb *Hin*dIII bands, were detected by the β_1-AR probe in DNA from Chinese hamster and hybrid cell lines.

In situ chromosomal hybridization has regionally localized the α_1- and β_1-AR genes to chromosomal regions 5q32–q34 and 10q24–q26, respectively (Fig. 1). Of 107 grains on 50 cells analyzed with the α_1-AR probe, 21 (18.7%) were located at 5q32–q34. For β_1-AR, of 107 grains on 55 metaphases scored, 17.8% (20) of the grains hybridized to 10q24→q26.

α_1-, α_2-, and β_1-AR Mapping in the Mouse. A panel of 12 Chinese hamster–mouse and one rat–mouse somatic cell hybrids was used to localize the α_1-AR gene to mouse chromosome 11. Three stronger *Pst* I/*Eco*RI mouse-specific hybridizing fragments of 1.9, 1.8, and 1.1 kb and one faint *Pst* I/*Eco*RI mouse 2.6-kb band were detected. The 1.9- and 1.1-kb mouse *Pst* I/*Eco*RI bands were present only in DNA from the rat–mouse hybrid containing mouse chromosome 11 and absent in DNA from all other hybrids, which did not retain mouse chromosome 11 (Table 2). The 2.6-kb mouse band is not scorable because of the limited hybridization sensitivity. The 1.8-kb mouse *Pst* I/*Eco*RI fragment was not present in any hybrids and probably represents a polymorphism.

Assignments of the mouse α_2- and β_1-AR loci to chromosome 19 were accomplished by Southern analysis of DNA from 15 Chinese hamster–mouse hybrids and one mouse–rat hybrid. The mouse-specific α_2-AR 3-kb *Pst* I fragment and the β_1-AR 6.4-kb *Bam*HI and 1.1-kb *Pst* I fragments were concordant only with mouse chromosome 19 (Table 2). Cross-hybridization fragments of different sizes compared with mouse-specific bands were detected by α_2- and β_1-AR probes in DNA from Chinese hamster and rat.

Pulsed Field Gel Electrophoresis. DNA samples from six individuals were digested with the following enzymes: *Sfi* I, *Sal* I, *Sac* II, *Pvu* I, *Mlu* I, and the combination of *Sfi* I with

Table 1. Correlation of human sequences detected by α_1- and β_1-AR probes with human chromosomes in Chinese hamster–human somatic hybrids

Presence of sequence/ presence of 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
α_1																							
Concordant																							
+/+	0	0	1	0	2	2	0	1	0	1	1	0	1	1	1	0	1	1	1	1	1	2	0
−/−	7	9	4	6	10	6	7	6	8	10	6	6	6	3	4	6	10	4	6	8	4	4	1
Discordant																							
+/−	2	2	1	1	0	0	2	1	2	1	1	2	1	1	1	2	1	1	1	1	1	0	1
−/+	4	2	6	3	0	5	2	5	3	1	3	5	5	6	7	4	1	6	5	3	6	6	4
Total discordant hybrids	6	4	7	4	0	5	4	6	5	2	4	7	6	7	8	6	2	7	6	4	7	6	5
Total informative hybrids	13	13	12	10	12	13	11	13	13	13	11	13	13	11	13	12	13	12	13	13	12	12	6
% discordant	46	31	58	40	0	38	36	46	38	15	36	54	46	64	62	50	15	58	46	31	58	50	83
β_1																							
Concordant																							
+/+	0	0	2	0	1	2	1	0	0	2	1	1	0	1	1	0	0	0	0	1	1	2	1
−/−	6	9	5	6	8	6	7	4	7	10	6	7	4	3	3	5	8	3	4	7	4	3	2
Discordant																							
+/−	2	2	0	0	1	0	1	2	2	0	1	1	2	1	1	2	2	2	2	1	1	0	0
−/+	4	1	5	3	1	4	1	6	3	0	2	3	6	5	7	4	2	7	6	3	5	6	2
Total discordant hybrids	6	3	5	3	2	4	2	8	5	0	3	4	8	6	8	6	4	9	8	4	6	6	2
Total informative hybrids	12	12	12	9	11	12	10	12	12	12	10	12	12	10	12	11	12	12	12	12	11	11	5
% discordant	50	25	42	33	18	33	20	67	42	0	30	33	67	60	67	55	33	75	67	33	55	55	40

Chromosomes with rearrangement or present at a frequency of 0.1 or less were excluded.

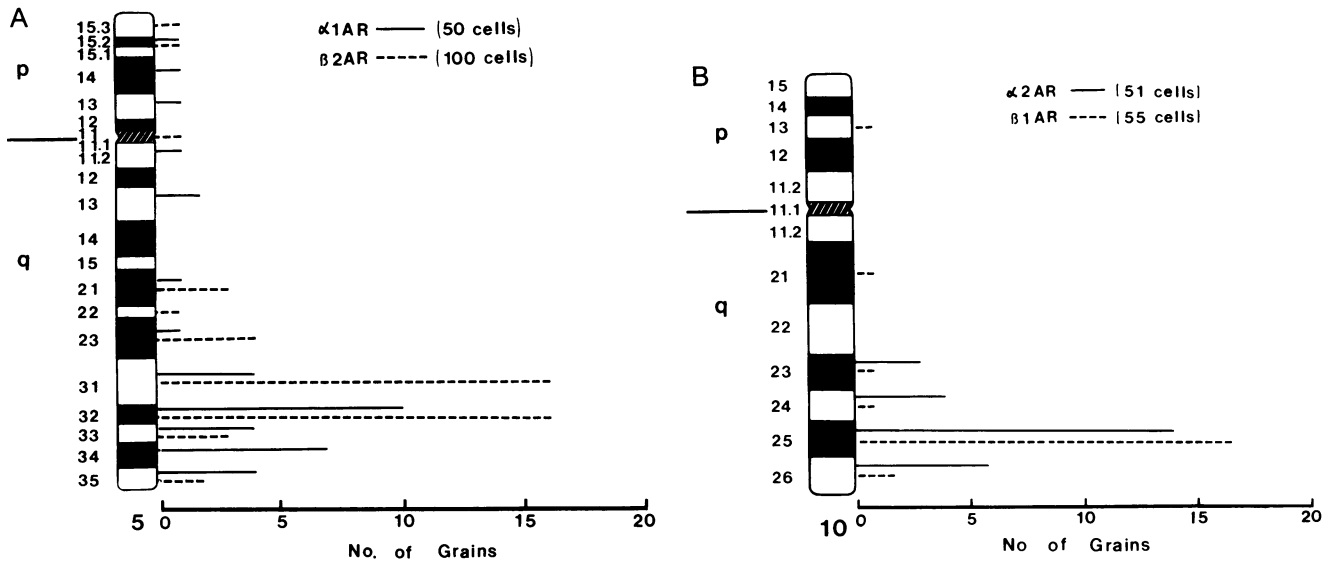

FIG. 1. Silver grain distribution along human chromosomes 5 (*A*) and 10 (*B*) after *in situ* hybridization with AR probes, illustrating the identical map position of the α_1- and β_2-AR loci on chromosome 5 (*A*) and the α_2- and β_1-AR loci on chromosome 10 (*B*).

Sac II, *Mlu* I or *Pvu* I. The size of hybridizing fragments was determined according to the λ multimers and yeast chromosome markers. The sizes of various restriction fragments detected by AR probes are summarized in Table 3. A 300-kb fragment was detected by α_1- and β_2-AR probes in samples digested by *Sfi* I alone or in combination with other enzymes (Fig. 2*A*). This 300-kb fragment could be a partially cut band of 120 kb and 150 kb of α_1- and β_2-AR genes, respectively. In that case, the α_1- and β_2-AR genes are likely <300 kb apart.

Both α_2- and β_1-AR probes hybridized to fragments of the same size in at least five digests. The smallest fragment that was common to both genes is 225 kb, which suggests that the distance between α_2- and β_1-AR loci is within 225 kb (Fig. 2*B*). All four AR probes detected a *Pvu* I fragment at ≥2000 kb, which is above the resolution of these gels.

DISCUSSION

As part of our continuing efforts to map AR genes to human and mouse chromosomes, we have assigned the α_1-AR gene to human chromosome 5 bands 5q32→q34 and the β_1-AR gene to human chromosome 10 bands 10q24→q26. The mouse α_2- and β_1-AR loci were localized to mouse chromosome 19 and the α_1-AR locus was mapped to mouse chromosome 11. Autosomal segments containing homologous gene loci have been conserved in widely divergent mamma-

Table 2. Correlation of mouse sequences detected by α_1-, α_2-, and β_1-AR probes with mouse chromosomes in Chinese hamster–mouse and mouse–rat somatic hybrids

Presence of sequence/ presence of chromosome	Mouse chromosome																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
α_1																				
Concordant																				
+/+	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
−/−	3	2	6	6	7	5	3	6	9	7	12	5	8	8	3	5	3	7	3	3
Discordant																				
+/−	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
−/+	8	10	6	5	3	5	8	6	3	4	0	7	3	4	9	6	9	4	8	8
Total discordant hybrids	9	11	7	6	4	6	9	7	4	5	0	8	3	5	10	7	10	5	9	9
Total informative hybrids	12	13	13	12	11	11	12	13	13	12	13	13	12	13	13	12	13	12	121	12
% discordant	75	85	54	50	36	55	75	54	31	42	0	62	25	38	77	58	77	42	75	75
α_2/β_1																				
Concordant																				
+/+	6	9	7	6	4	5	7	7	2	3	0	5	3	4	8	5	8	4	9	6
−/−	3	5	6	7	6	5	3	7	5	5	6	4	5	5	3	2	5	6	6	2
Discordant																				
+/−	3	0	2	2	4	2	1	2	7	5	9	4	5	5	1	3	1	4	0	2
−/+	2	2	1	0	0	1	4	0	1	2	1	3	1	1	4	5	2	1	0	5
Total discordant hybrids	5	2	3	2	4	3	5	2	8	7	10	7	6	6	5	8	3	5	0	7
Total informative hybrids	14	16	16	15	14	13	15	16	15	15	16	16	14	15	16	15	16	15	15	15
% discordant	36	13	19	13	29	23	33	13	53	47	63	44	44	40	31	53	19	33	0	47

Chromosomes with rearrangement or present at a frequency of 0.1 or less were excluded.

Table 3. Fragments (in kb) detected with AR probes by pulsed field gel electrophoresis

Enzyme	α_1	β_2	α_2	β_1
Sfi I	120, 300	150, 300, 550, 700	225	225
Sal I	250, 450, 550, 680, 730, >2000	370, 730, 850, >1000	380, 510	380, 510
Sac II	70, 300	>2000	>2000	>2000
Pvu I	>2000	>2000	>2000	>2000
Mlu I	1100, >2000	>2000	>2000	>2000
Sfi I/*Sac* II	120, 300, >2000	150, 300, 550, 700, >1000	225	225, 145
Sfi I/*Mlu* I	120, 300, >2000	150, 300, 550, 700, >1000	225	225, 145
Sfi I/*Pvu* I	120	150	225	225, 145

lian species. Their identification and delineation reveal the chromosomal events that have occurred during evolution. Homologous genes located on the distal long arm of human chromosome 5 are on mouse chromosomes 11 and 18 (10, 18, 19). Both α_1- and β_2-AR genes are on human distal 5q, but the α_1-AR gene belongs to the conserved syntenic group on mouse chromosome 11, which includes gene encoding granulocyte–macrophage colony-stimulating factor, interleukins 3, 4, and 5, and acidic cysteine-rich secreted protein (20, 21), and the β_2-AR gene joins colony-stimulating factor 1 receptor, glucocorticoid receptor, and platelet-derived growth factor receptor genes on mouse chromosome 18 (10, 18, 19). It is currently impossible to separate the cluster of loci syntenic with mouse chromosome 11 or 18 on human distal 5q since the order of these genes has not been precisely established.

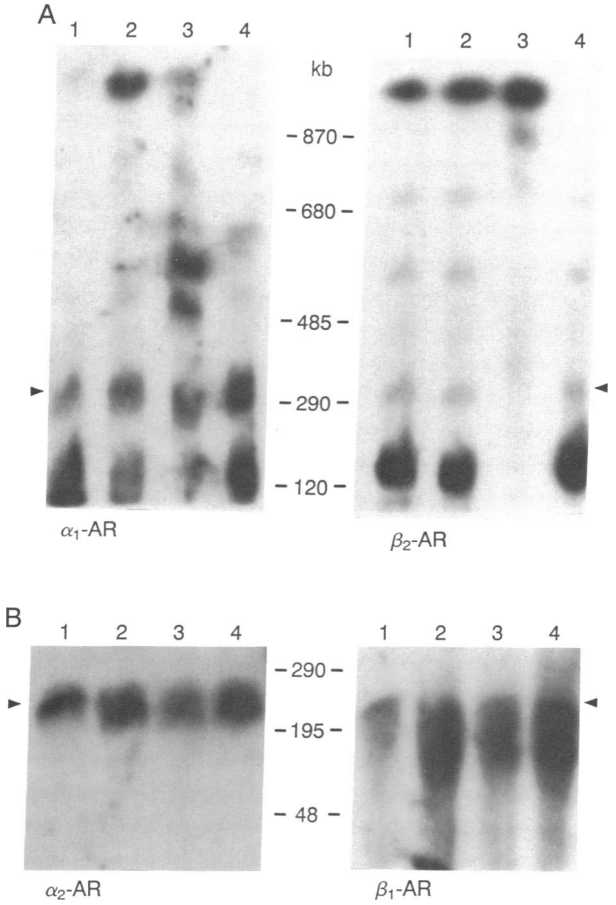

FIG. 2. Pulsed field gel electrophoresis analysis of the AR genes. (*A*) DNA samples were digested with *Sfi* I/*Sac* II (lane 1), *Sfi* I/*Mlu* I (lane 2), *Sal* I (lane 3), and *Sfi* I (lane 4) and were separated by pulsed field gel electrophoresis. A Southern blot of this gel was hybridized with α_1-AR and β_2-AR probes sequentially. Arrows indicate the common fragment detected by both probes. (*B*) DNA samples were digested with *Sfi* I/*Sac* II (lane 1), *Sfi* I/*Mlu* I (lane 2), *Sfi* I/*Pvu* I (lane 3), and *Sfi* I (lane 4) and were separated by pulsed field gel electrophoresis. A Southern filter of this gel was hybridized with α_2-AR and β_1-AR probes sequentially. Arrows indicate the fragment corecognized by both probes.

The chromosomal region 10q24→q26 in which the α_2- and β_1-AR genes are located is part of a known segment with genes encoding terminal deoxynucleotidyltransferase, lipase A, glutamic oxaloacetic transaminase, phosphoglyceromutase 1, and cytochrome P-450 that are conserved on human chromosome 10 and mouse chromosome 19 (18, 19, 22). Assignment of the murine α_2- and β_1-AR genes to chromosome 19 adds two more genes to this conserved syntenic group.

Comparative mapping in humans and mice advances our understanding of mammalian genome organization and its evolution. The AR genes are distinct from each other, but their significant sequence homology and similar structural features suggest that they are evolutionarily related. The α_2- and β_1-AR genes located on the same chromosome in human and mouse likely arose from an ancestral gene duplication. The α_1- and β_2-AR loci on human 5q may have arisen by chromosomal duplication. The separation of the α_1- and β_2-AR loci in the mouse could result from additional chromosomal rearrangement in the mammalian lineage leading to the mouse.

The α_1-AR gene was assigned to the same band position as the β_2-AR gene on human chromosome 5q32→q34 (9) and the β_1-AR gene was regionally localized to human chromosome 10q24→q26, which coincides with the site on 10q detected by the α_2-AR probe (8). Although these two pairs of genes were mapped to the same chromosomal bands by *in situ* hybridization, the region where they are situated covers at least 3000–6000 kb because of the limited resolution at the cytological level. Pulsed field gel electrophoresis has further localized the α_1- and β_2-AR genes to within 225 kb, and the α_2- and β_1-AR genes to within 300 kb. The close linkage of structurally and functionally related genes has been demonstrated previously for hemopoietic growth factor genes. Granulocyte–macrophage colony-stimulating factor and interleukin 3 were found to be 9 kb apart in the human genome (23) and 14 kb apart in the mouse genome (22). The human colony-stimulating factor 1 receptor and platelet-derived growth factor receptor genes are linked in a tandem fashion and are separated by <500 bp (17). There may have been some common evolutionary steps for the AR and hemopoietic growth factor gene families because of the similarities in their function (as receptors) as well as their physical proximity in the genome.

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1. Lefkowitz, R. L., Stadel, J. M. & Caron, M. G. (1983) *Annu. Rev. Biochem.* **52,** 159–186.
2. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56,** 615–649.
3. Findlay, J. B. C. & Pappin, D. J. C. (1986) *Biochem. J.* **238,** 625–642.
4. Kobilka, B. K., Kobilka, T. S., Daniel, K., Regan, J. W., Caron, M. G. & Lefkowitz, R. J. (1988) *Science* **240,** 1310–1316.
5. Dohlman, H. G., Caron, M. G. & Lefkowitz, R. J. (1987) *Biochemistry* **26,** 2657–2664.

6. Kobilka, B. K., Frielle, T., Collins, S., Yang-Feng, T., Kobilka, T. S., Francke, U., Lefkowitz, R. J. & Caron, M. G. (1987) *Nature (London)* **329,** 75–79.
7. Fargin, A., Raymond, J. R., Lohse, M. J., Kobilka, B. K., Caron, M. G. & Lefkowitz, R. J. (1988) *Nature (London)* **335,** 358–360.
8. Kobilka, B. K., Matsui, H., Kobilka, T. S., Yang-Feng, T. L., Francke, U., Caron, M. G., Lefkowitz, R. J. & Regan, J. W. (1987) *Science* **238,** 650–656.
9. Kobilka, B. K., Dixon, R. A., Frielle, T., Dohlman, H. G., Bolanowski, M. A., Sigal, I. S., Yang-Feng, T. L., Francke, U., Caron, M. G. & Lefkowitz, R. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 46–50.
10. Sundaresan, S., Francke, U. (1989) *Somat. Cell Mol. Genet.* **15,** 367–371.
11. Cotecchia, S., Schwin, D. A., Randall, R. R., Lefkowitz, R. J., Caron, M. G. & Kobilka, B. K. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7159–7163.
12. Frielle, T., Collins, S., Daniel, K. W., Caron, M. G., Lefkowitz, R. J. & Kobilka, B. K. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7920–7924.
13. Yang-Feng, T. L., Landau, N. R., Baltimore, D. & Francke, U. (1986) *Cytogenet. Cell Genet.* **43,** 121–126.
14. Feinberg, A. R. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
15. Herrmann, B. G., Barlow, D. P. N. & Lehrach, H. (1987) *Cell* **48,** 813–825.
16. Chu, G., Vollrath, D. & Davis, R. W. (1986) *Science* **234,** 1582–1585.
17. Roberts, W. M., Look, T., Roussel, M. F. & Sherr, C. J. (1988) *Cell* **55,** 655–661.
18. Searle, A. G., Peters, J., Lyon, M. F., Hall, J. G., Evans, E. P., Edwards, J. H. & Buckle, V. J. (1989) *Ann. Hum. Genet.* **53,** 89–140.
19. Lalley, P. A., O'Brien, S. J., Creau-Goldberg, N., Davison, M. T., Roderick, T. H., Echard, G., Womack, J. E., Graves, J. M., Doolittle, D. P. & Guidi, J. N. (1987) *Cytogenet. Cell Genet.* **46,** 367–368.
20. Barlow, D. P., Bucan, M., Lehrach, H., Hogan, B. L. M. & Gough, N. M. (1987) *EMBO J.* **6,** 617–623.
21. Ihle, J. N., Silver, J. & Kozak, C. A. (1987) *J. Immunol.* **138,** 3051–3054.
22. Lee, J. S. & Young, I. G. (1989) *Genomics* **5,** 359–362.
23. Yang, Y.-C., Kovacic, S., Kriz, R., Wolf, S., Clark, S. C., Wellems, T. E., Nienhuis, A. & Epstein, N. (1988) *Blood* **71,** 958–961.