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Chromosomal Assignment of Retinoic Acid Receptor (*RAR*) Genes in the Human, Mouse, and Rat Genomes

MARIE-GENEVIÈVE MATTEI,* MICHÈLE RIVIÈRE,† ANDRÉE KRUST,‡ SIGURDUR INGVARSSON,§ BJÖRN VENNSTRÖM,§ M. QUAMRUL ISLAM,|| GÖRAN LEVAN,|| PHILIPPE KAUTNER,‡ ARTHUR ZELENT,‡ PIERRE CHAMBON,‡ JOSIANE SZPIRER,† AND CLAUDE SZPIRER†

*INSERM U 242, Hôpital d'Enfants de la Timone, Centre de Génétique Médicale, F-13385 Marseille, France; †Université Libre de Bruxelles, Département de Biologie Moléculaire, Rue des Chevaux, 67, B-1640 Rhode-St-Genèse, Belgium; ‡INSERM U184 and CNRS, LGME, Institut de Chimie Biologique, Faculté de Médecine, Rue Humann, 11, F-67085 Strasbourg, France; §Karolinska Institute, Department of Molecular Biology, Box 60400, S-104 01 Stockholm, Sweden; and ||University of Gothenburg, Department of Genetics, Box 33031, S-40033 Gothenburg, Sweden

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The human genes encoding the α and β forms of the retinoic acid receptor are known to be located on chromosomes 17 (band q21.1: *RARA*) and 3 (band p24: *RARB*). By *in situ* hybridization, we have now localized the gene for retinoic acid receptor γ, *RARG*, on chromosome 12, band q13. We also mapped the three retinoic acid receptor genes in the mouse, by *in situ* hybridization, on chromosomes 11, band D (*Rar-a*); 14, band A (*Rar-b*); and 15, band F (*Rar-g*), respectively, and in the rat, using a panel of somatic cell hybrids that segregate rat chromosomes, on chromosomes 10 (*RARA*), 15 (*RARB*), and 7 (*RARG*), respectively. These assignments reveal a retention of tight linkage between *RAR* and *HOX* gene clusters. They also establish or confirm and extend the following homologies: (i) between human chromosome 17, mouse chromosome 11, and rat chromosome 10 (*RARA*); (ii) between human chromosome 3, mouse chromosome 14, and rat chromosome 15 (*RARB*); and (iii) between human chromosome 12, mouse chromosome 15, and rat chromosome 7 (*RARG*).

INTRODUCTION

The retinoic acid receptors (RAR) are transcriptional enhancer factors, as well as members of the thyroid/steroid hormone receptor family (Giguere *et al.*, 1987; Petkovitch *et al.*, 1987; Brand *et al.*, 1988). Retinoic acid is a developmental signaling molecule and can modulate the differentiation of many types of cells (Roberts and Sporn, 1984; Eichele, 1989; Summerbell and Maden, 1990). Three retinoic acid receptor subtypes (α, β, γ, corresponding to the genes *RARA, RARB,* and *RARG,* respectively) have been identified in man and mouse (Zelent *et al.*, 1989; Krust *et al.*, 1989). The gene encoding the RARα form (*RARA*) gene is rearranged in acute promyelocytic leukemia cells (Borrow *et al.*, 1990; de Thé *et al.*, 1990), and the RARβ (*RARB*) gene has been shown to be a site for hepatitis B virus integration in one hepatocellular carcinoma (Dejean *et al.*, 1986; de Thé *et al.*, 1987). Altered *RAR* genes thus seem to have oncogenic properties.

The localizations of the human *RARA* and *RARB* genes are known (17q21.1 and 3p24, respectively) (Mattei *et al.*, 1988a,b). We report here the localization of the third human gene, *RARG,* encoding the receptor γ (on chromosome 12, band q13), and of the three *RAR* genes in the mouse and the rat genomes.

MATERIALS AND METHODS

Mapping by in Situ Hybridization

Chromosome spreads preparation. *In situ* hybridizations were carried out on metaphase chromosomes spreads. These were obtained from phytohemagglutinin-stimulated human lymphocytes cultured for 72 h or from concavanalin A-stimulated mouse lymphocytes from a WMP/Pas inbreed strain male in which all autosomes except chromosome 19 are in the form of metacentric Robertsonian translocations. To ensure a posthybridization chromosomal banding of good quality, 5-bromodeoxyuridine was added for the final 7 (human) or 6 (mouse) h of culture (60 μg/ml of medium).

Probe preparation. All the probes were tritium-labeled by nick-translation to a specific activity of 2×10^8 dpm/μg. The probes used were the entire human cDNA insert, designated hRARγ (Krust *et al.*, 1989), and the three entire mouse cDNAs, α, β, and γ, designated mRARα, mRARβ, and mRARγ, respectively

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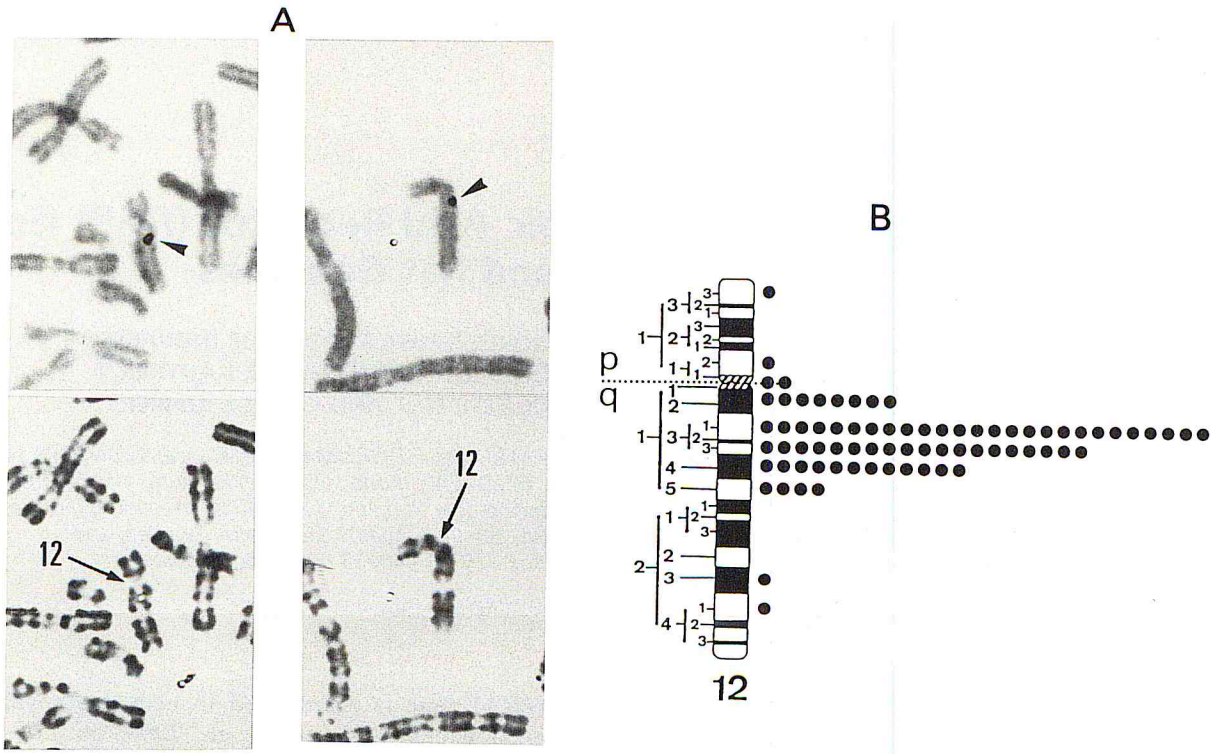

FIG. 1. Assignment of the human RARγ locus (*RARG*) to chromosome 12 by *in situ* hybridization. (**A**) Two partial human metaphases showing the specific sites of hybridization. (**Top**) Arrowheads point to silver grains on Giemsa-stained chromosomes after autoradiography. (**Bottom**) Same metaphases, but R-banded (FPG method) and the labeled region of chromosome 12 can be identified. (**B**) Idiogram of the human G-banded chromosome 12 showing the detailed distribution of labeled sites. One hundred metaphase cells were examined for the presence of silver grains associated with chromosomes. A total of 158 grains was scored, 75 of those (47,4%) were found to be associated with chromosome 12 and the great majority (76%) of them mapped to region 12q13.1–q14 with a maximum in the q13 band. There was a second significant cluster of silver grains (12,6% of the total) associated with chromosome 17 in the proximal part of band q21 (data not shown).

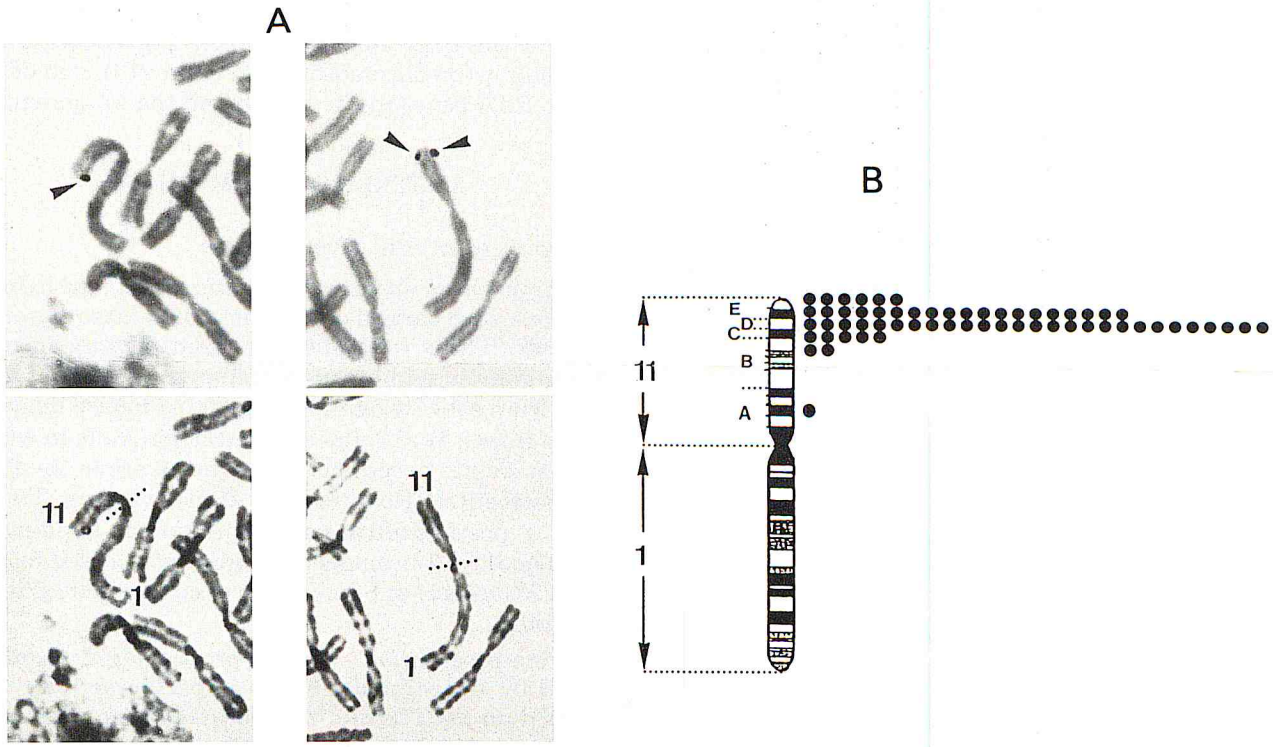

FIG. 2. Assignment of the mouse RARα locus (*Rar-a*) to mouse chromosome 11 by *in situ* hybridization. (**A**) Two partial WMP mouse metaphases showing the specific site of hybridization. (**Top**) Arrowheads point to silver grains on Giemsa-stained chromosomes after autoradiography. (**Bottom**) Chromosomes with silver grains subsequently identified by R-banding. (**B**) B-band diagram illustrating the detailed distribution of labeled sites. Of 126 silver grains on 100 metaphase cells analyzed, 60 (47.6%) were located on chromosome 11. Most of the grains (76.6%) are regionally localized in the D–E1 region with a maximum in the D band.

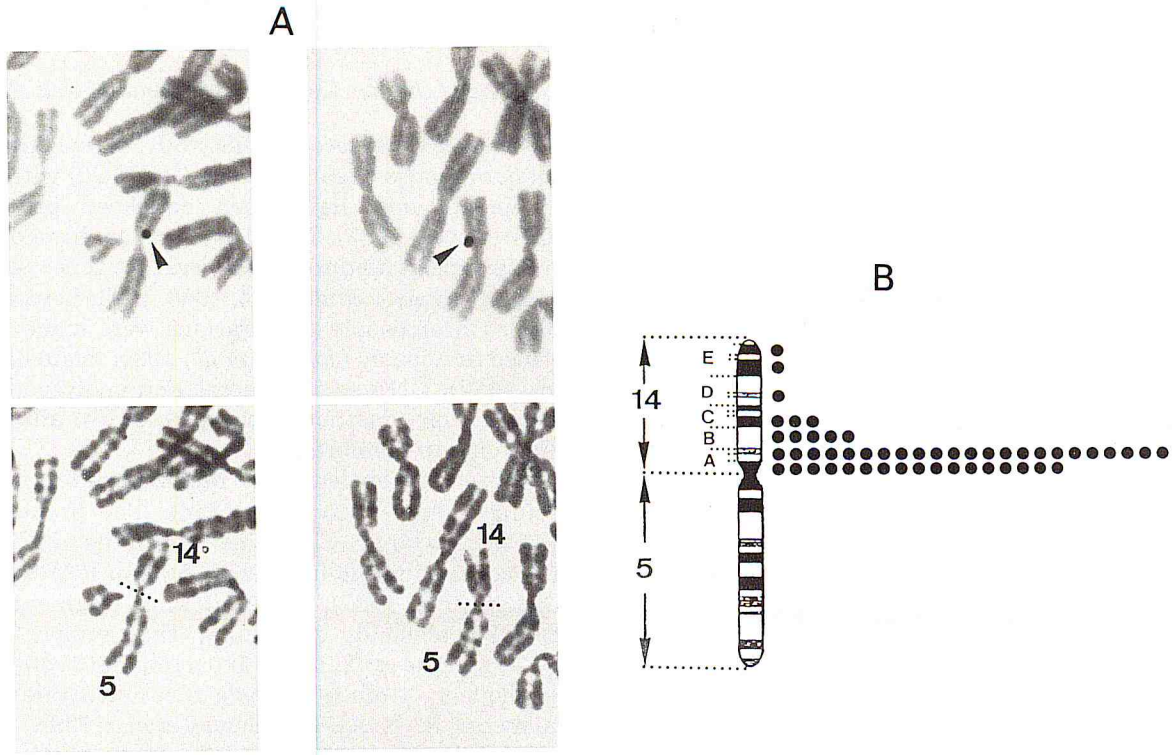

FIG. 3. Assignment of the mouse RARβ locus (*Rar-b*) to mouse chromosome 14 by *in situ* hybridization. (**A**) Two partial WMP mouse metaphases showing the specific site of hybridization. (**Top**) Arrowheads indicate silver grains on Giemsa-stained chromosomes after autoradiography. (**Bottom**) Chromosomes with silver grains subsequently identified by R-banding. (**B**) G-band diagram of chromosome 14 illustrating the distribution of labeled sites. Of 100 metaphase cells examined, 128 silver grains were associated with chromosomes and 51 (39.8%) of them were located on chromosome 14 and 78.4% of these mapped to the A1–A3 region of chromosome 14.

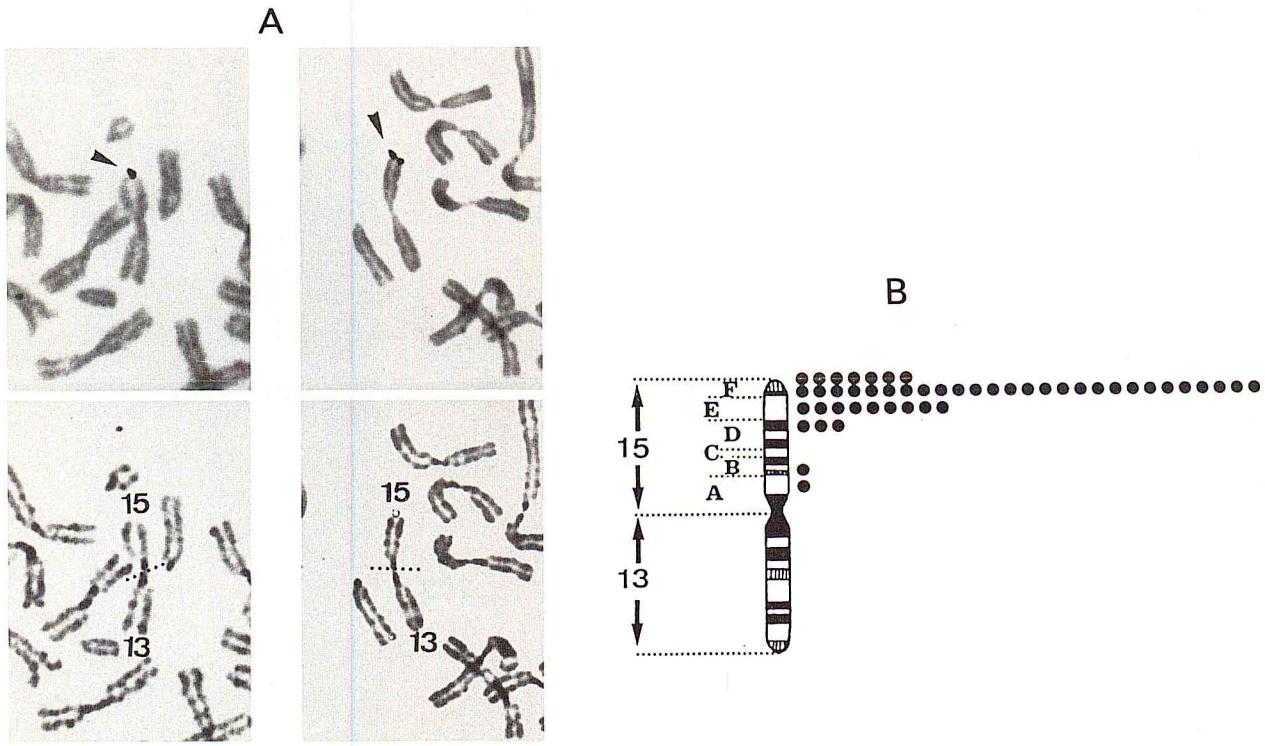

FIG. 4. Assignment of the mouse RARγ locus (*Rar-g*) to chromosome 15 by *in situ* hybridization. (**A**) Two partial WMP mouse metaphases showing the specific site of hybridization. (**Top**) Arrowheads indicate silver grains on Giemsa-stained chromosomes after autoradiography. (**Bottom**) Chromosomes with silver grains subsequently identified by R-banding. (**B**) G-band diagram locating the grains hybridized on chromosome 15. In the 100 metaphase cells examined 41.3% of the silver grains (48 of 116) associated with chromosomes were located on chromosome 15; 89.5% of them can be identified in region E–F3 with a major hybridization peak in the band F.

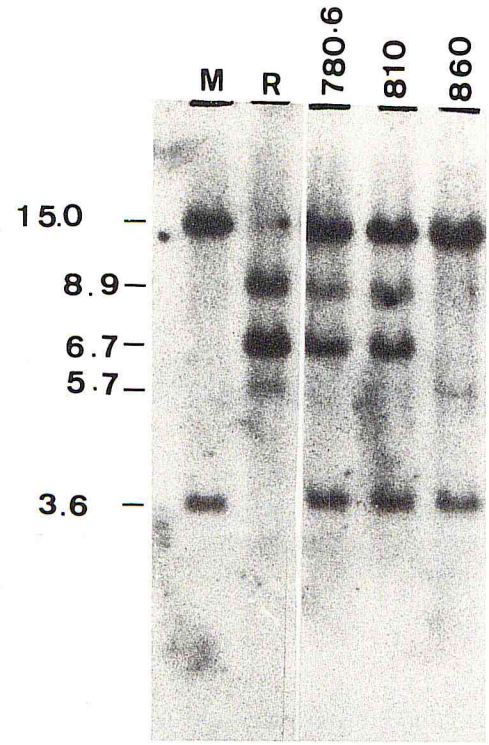

FIG. 5. Autoradiogram of a Southern blot of *Bam*HI-digested parental and mouse × rat cell hybrids, hybridized with the human RARα probe. M, mouse (BWTG3) DNA; R, Sprague–Dawley rat DNA; the other three lanes correspond to three LB hybrids. The two rat-specific bands at 8.9 and 6.7 kb cosegregated with rat chromosome 10. However, a third and faint rat-specific band, visible at 5.7 kb, did not segregate with the other two rat fragments (as illustrated in this figure, LB780.6 is positive for the 8.9- and 6.7-kb fragments only, LB810 is positive for the three fragments, and LB860 is positive for the 5.7-kb fragment only). The rat 5.7-kb fragment segregated with rat chromosome 7, as the *RARG* gene (see text and Fig. 7) and is probably derived from this gene (in Fig. 7, this putative *RARG* fragment is not detected; this can easily be explained by the fact that the *RARG* probe used was not a full-length cDNA, unlike the *RARA* probe used here).

(Zelent *et al.*, 1989). All inserts were subcloned in the pSG5 vector (Green *et al.*, 1988).

In situ hybridization. The radiolabeled probes were hybridized to metaphase spreads at a final concentration of 25 ng/ml of hybridization solution as previously described in Mattei *et al.* (1985).

Autoradiography, staining, and banding. After coating with nuclear track emulsion, the slides were exposed for 24 days (hRARγ), 15 days (mRARα and mRARγ), or 20 days (mRARβ) at +4°C. To avoid any slipping of silver grains during the banding procedure, chromosome spreads were first stained with buffered Giemsa solution and metaphases photographed. R-banding was then performed by the fluorochrome–photolysis–Giemsa (FPG) method and metaphases were rephotographed before analysis.

Mapping of the Rat Genes Using Somatic Cell Hybrids

The cell hybrids used in this study, derived from the fusion of mouse hepatoma cells (BWTG3) with adult rat hepatocytes, have been described previously (Szpirer *et al.*, 1984). They have lost rat chromosomes and have been used to map several rat genes (see, for instance, Szpirer *et al.*, 1984, 1988, 1991; Levan *et al.*, 1990). Chromosome preparations were made as described previously (Szpirer *et al.*, 1984; Islam and Levan, 1987). DNA was extracted and analyzed by the Southern blot method (Southern, 1975), after blotting to nylon membranes.

Probes were labeled by the random priming method (Feinberg and Vogelstein, 1983). The probes used were the 2.9-kb *Eco*RI fragment from the pHK1 plasmid, containing the full-length human RARα cDNA (Giguere *et al.*, 1987); the 1.4-kb *Mae*I fragment of the pCOD20 plasmid, containing the human RARβ cDNA (de Thé *et al.*, 1987); and a fragment containing the sequence from nucleotide 1 to nucleotide 534 of the mouse RARγ cDNA (Zelent *et al.*, 1989).

Hybridizations were carried out at 65°C, in 3× SSC, 10× Denhardt's solution, in the presence of salmon sperm DNA (150 μg/ml), with probes at a concentration of 2–3 ng/ml; with the two probes giving a high background (*RARA* and *RARB* genes), these

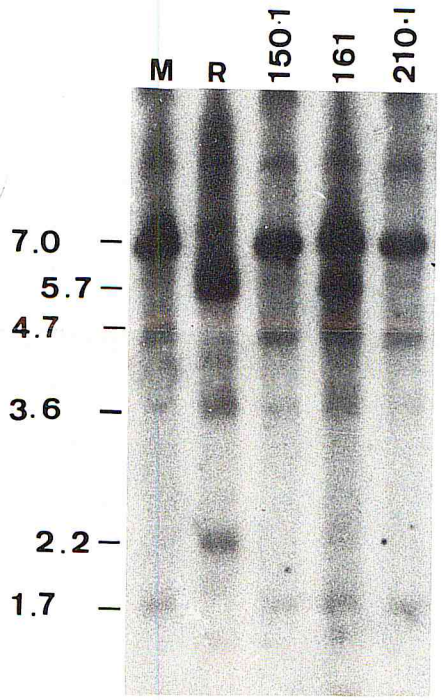

FIG. 6. Autoradiogram of a Southern blot of *Hin*dIII-digested parental and mouse × rat cell hybrids, hybridized with the human RARβ probe. M, mouse (BWTG3) DNA; R, Sprague–Dawley rat DNA; the other three lanes correspond to three LB hybrids.

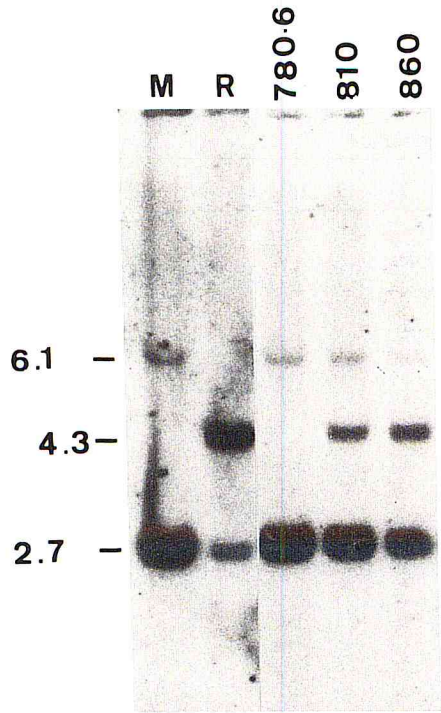

FIG. 7. Autoradiogram of a Southern blot of *Bam*HI-digested parental and mouse × rat cell hybrids, hybridized with the mouse RARγ probe. M, mouse (BWTG3) DNA; R, Sprague–Dawley rat DNA; the other three lanes correspond to three LB hybrids.

concentrations were modified to 300 μg/ml and 0.5 ng/ml, respectively, and in the case of the *RARB* probe, rodent DNA was also added in the hybridization mixture (15 μg/ml of rat DNA).

RESULTS

The Human RARG Gene

In the 100 metaphases cells examined after *in situ* hybridization, there were 158 silver grains associated with chromosomes and 75 of these (47.4%) were located on chromosome 12. The distribution of grains on this chromosome was not random: 76% (57/75) of them mapped to the q13.1–q14 region of the chromosome 12 long arm, with a maximum in the q13 band (Fig. 1). A secondary hybridization site was reproducibly detected on chromosome 17, which clustered 12.6% (20/158) of total silver grains. The grain distribution on this chromosome showed a significant peak (80%) in the proximal part of the 17q21 band, i.e., the position of the *RARA* gene. This cross-hybridization could be due to sequence homology between the *RARG* and *RARA* genes. Nevertheless, these results allow us to map the human *RARG* gene to the q13 band of chromosome 12.

The Three Mouse Rar Genes

For each of the three genes, 100 metaphase cells were examined after *in situ* hybridization. In the case of the *Rar-a* gene, there were 126 silver grains associated with chromosomes, 60 (47.6%) of which were located on chromosome 11; 76% (46/60) of the grains mapped to the D–E1 region of chromosome 11, with a maximum in the D band (Fig. 2). This result allows us to assign the *RARa* gene to the 11D band of the mouse genome.

The *Rar-b* gene probe showed 128 grains associated with chromosomes, 51 (39.8%) of which were located on chromosome 14; 78% (40/51) of them mapped to the A1–A3 region of this chromosome (Fig. 3). We thus conclude that the *Rar-b* gene maps in the 14A band of the mouse genome.

Finally, in the case of the *Rar-g* gene probe, there were 116 silver grains associated with chromosomes and 48 of these (41.3%) were located on chromosome 15; as in the three previous analyses, the distribution of grains was not random: 89% (43/48) of the grains mapped to the E–F3 region of chromosome 15, with a maximum in the F band (Fig. 4). As in the case of the human *RARG* gene, a minor peak was reproducibly detected on another chromosome, namely, chromosome 11, with 9.5% of total silver grains. The grain distribution on this chromosome showed a significant cluster in the 11D band, the position of the *Rar-a* gene (see above). The most probable localization of the mouse *Rar-g* gene is thus the F band of chromosome 15.

The Three Rat RAR Genes

The assignment of the three rat *RAR* genes was determined by Southern blot analysis of a series of well-characterized mouse × rat cell hybrids. After digestion with an adequate restriction enzyme (i.e., allowing the unambiguous detection of rat-specific fragments), the presence of each of the rat genes could be determined in the DNA from these hybrids. *Bam*HI was used in the case of *RARA;* the main rat-specific restriction fragments were detected at 8.9 and 6.7 kb (Fig. 5). *Hin*dIII was used in the case of *RARB;* rat-specific restriction fragments were visible at 5.7 and 2.2 kb (Fig. 6). *Bam*HI was also used in the case of *RARG;* a rat-specific 4.3-kb restriction fragment was detected (Fig. 7). The results obtained are summarized in Table 1, where the segregation of the three rat *RAR* genes is compared with the rat chromosome composition of the hybrids (in each case the different rat fragments mentioned above cosegregated in the hybrids). Each rat gene segregated clearly with one specific rat chromosome: no discordant hybrid was found for *RARA* and rat chromosome 10, for *RARB* and rat chromosome 15, and for *RARG* and rat chromosome 7. Several discordant hybrids (at least three)

TABLE 1

Mouse × Rat Hybrids: Presence of the Rat *RAR* Genes and Rat Chromosome Content

	Rat *RAR* genes[a]			Rat chromosomes[b]																			
	A	B	G	X	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Hybrids																								
LB20	N	−	+	+	−	(+)	(+)	−	−	−	+	−	−	−	−	+	+	−	−	+	(+)	+	+	−
LB150-1	+	−	+	+	−	−	+	+	−	−	+	−	+	(−)	+	(+)	+	−	−	(+)	(+)	+	(+)	−
LB161	+	+	+	+	−	+	+	+	+	+	+	−	+	+	−	(−)	+	+	+	+	+	+	+	(+)
LB210-I	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+	−	−
LB251	+	−	+	+	+	+	−	+	−	(+)	+	−	−	+	−	+	+	−	−	−	+	−	+	−
LB330	N	−	−	+	−	+	+	+	−	+	−	−	−	+	−	+	−	−	−	−	+	−	−	−
LB330TG3	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
LB330TG6	+	−	−	−	−	+	−	+	−	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−
LB510-6	−	+	+	+	−	+	+	+	−	−	+	−	−	−	−	+	+	+	+	+	+	+	−	−
LB600	+	+	+	+	+	+	+	+	+	(+)	+	−	(−)	+	+	+	+	+	+	+	−	+	+	−
LB630	−	+	+	+	(−)	−	+	+	(+)	+	+	−	+	−	+	+	+	(+)	+	+	−	+	+	(−)
LB780-6	+	−	−	+	−	+	−	−	−	−	−	−	−	+	+	−	+	−	−	−	−	+	−	−
LB780-8	N	N	+	+	−	+	−	−	−	−	+	−	−	+	+	−	−	−	−	−	−	+	−	−
LB810	+	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	−	+	(+)
LB860	−	+	+	+	−	+	+	+	−	−	+	−	+	−	+	+	+	−	+	+	+	+	−	(+)
LB1040TG1	−	N	−	−	−	−	−	+	−	+	−	−	−	−	+	+	−	−	+	+	−	+	−	−
LB1040TG3	−	N	(−)	−	−	−	−	+	−	+	(−)	−	−	−	+	−	−	−	+	+	−	+	−	−
LB1040TG5	+	+	−	−	−	−	−	+	−	+	−	−	−	(+)	+	−	−	−	(+)	+	−	+	−	−
Independent discordant clones[c]																								
RARA				6	6	4	7	6	6	5	5	7	7	0	6	6	6	8	8	8	5	8	4	7
RARB				7	6	6	4	3	4	4	3	6	4	6	4	5	6	3	0	3	7	5	6	3
RARG				3	7	4	3	4	7	7	0	9	5	6	6	3	3	6	5	4	4	5	3	6

[a] + and −, presence or absence of rat hybridization signal, respectively; (−), weak rat hybridization signal; N, not done.

[b] +, rat chromosome present in more than 55% of the metaphases; (+), rat chromosome present in 25 to 55% of the metaphases; (−), rat chromosome present in less than 25% of the metaphases; −, rat chromosome absent.

[c] Independent hybrid clones are clones derived from distinct fusion events. They are identified by distinct numbers (nonindependent clones are, for instance, LB330TG3 and LB330TG6). When a chromosome was present in less than 25% of the metaphases (− in parentheses), the hybrid in question was not taken account to establish the number of discordances for that particular chromosome.

were obtained for each of the other combinations. In conclusion, the rat *RARA* resides on chromosome 10, the rat *RARB* resides on chromosome 15, and the rat *RARG* gene is located on chromosome 7.

As shown in Fig. 5, the *RARA* probe used also detected a 5.7-kb rat restriction fragment, rather weakly labeled, that did not segregate with the other rat fragments and with rat chromosome 10, but segregated with rat chromosome 7, which carries the *RARG* gene. This suggests that the probe used cross-hybridized with a *RARG* gene-derived restriction fragment. It is striking that in the *in situ* hybridization experiments, the human and mouse *RARG* probes hybridized with secondary sites corresponding to the position of the *RARA* genes. These observations suggest that RARα and RARγ sequences cross-hybridize more easily than any other pair of RAR sequences.

DISCUSSION

Table 2 summarizes our results and also shows the localization of some other genes to emphasize the relevant homologies between the human, mouse, and rat gene chromosome maps. It is clear that the *RARG* gene is not linked to the two other *RAR* genes and maps to human chromosome 12 and mouse chromosome 15, as previously mentioned (Krust *et al.*, 1989). Ishikawa *et al.* (1990) have recently reported the assignment of the human *RARG* gene to human chromosome 12.

The assignment of the *RARA* gene to mouse chromosome 11 and rat chromosome 10 confirms and extends the homology established between these two chromosomes on the one hand and with human chromosome 17 on the other hand (Szpirer *et al.*, 1988, 1991; Lalley *et al.*, 1989; Buchberg *et al.*, 1989; Nadeau and Reiner, 1989; Searle *et al.*, 1989; Levan *et al.*, 1991). Since the human *RARA* is altered in some tumors (Borrow *et al.*, 1990; de Thé *et al.*, 1990) and since translocations involving rat chromosome 10 have been described in rat hepatomas and mesotheliomas (Kovi *et al.*, 1978; Libbus and Craighead, 1988), it might be interesting to test these types of tumors for possible rearrangements of the *RARA* gene.

TABLE 2

Comparative Mapping

Locus: Human symbol	Chromosome location		
	Human (HSA)	Mouse (MMU)	Rat (RNO)
Retinoic receptor α: *RARA*	17 q21.1	11 D	10
Homeobox-2: *HOX2*	17 q21–q22	11 D	—
Thyroid hormone receptor α: *THRA1* (*ERBA1*)	17 q11.2–q12	11	10
AEV oncogene homolog 2: *ERBB2* (rat *neu*)	17 q11.2–q12	11 dist.	10
Retinoic acid receptor β: *RARB*	3 p24	14 A	15
Thyroid receptor β: *THRB* (*ERBA2*)	3 p24.1–p22	—	15
Retinoblastoma gene: *RB1*	13 q14.2	14	15
Retinoic acid receptor γ: *RARG*	12 q13	15 F	7
Homeobox-3: *HOX3*	12 q12–q13	15 F	—
Phenylalanine hydroxylase: *PAH*	12 q22–24	10	7
MYC oncogene	8 q24	15 D	7

Note. This table summarizes the localization in man, mouse, and rat of the genes tested in this work and of some other markers used to compare human, mouse, and rat chromosomes. For the references, see text, and for reviews, see Searle *et al.* (33), Lalley *et al.* (20), Nadeau and Reiner (29), and Levan *et al.* (22, 23).

Table 2 also shows that the *RARB* (*Rar-b*) gene is the second marker assigned to both mouse chromosome 14 and rat chromosome 15, the first one being the retinoblastoma (RB1) gene (Stone *et al.*, 1989; Szpirer *et al.*, 1991). These two genes thus define a new conserved synteny group in the two species. However, this synteny group is not retained in man.

Like the *RARB* gene, the *THRB* (*ERBA2*) maps on human chromosome 3 and on rat chromosome 15 (Dobrovic *et al.*, 1988; Drabkin *et al.*, 1988; Szpirer *et al.*, 1991). These two genes thus probably define a new synteny group conserved in one rodent species, rat, and in man. Our results identify the first gene, *RARB* (*Rar-b*), located both in man, on chromosome 3, and in mouse, on chromosome 14. The mouse *Thrb* (*Erba-2*) has not been localized, and it would be interesting to determine whether it is also located on chromosome 14 (probably in the band A). In the affirmative, this would extend the conservation of the *RARB–THRB* (*ERBA2*) synteny group to the mouse.

As also summarized in Table 2, the assignment of the *RARG* gene to human chromosome 12 and to rat chromosome 7 suggests that a new synteny group, comprising *RARB* and *PAH*, is retained in man on chromosome 12 and in rat on chromosome 7 (for the assignments of *PAH*, see Lidsky *et al.*, 1985; Fulchignoni-Lataud *et al.*, 1990). As already mentioned, another part of rat chromosome 7 (comprising the *MYC* and *TG* genes, for instance, Levan *et al.*, 1991) is already known to be homologous to human chromosome 8). With regard to the comparison with the mouse genome, rat chromosome 7 is highly homologous to mouse chromosome 15 (Levan *et al.*, 1991) (see also Table 2). Interestingly, the synteny group comprising *RARG* and *PAH*, conserved in man (12q) and rat (7), is not conserved in the mouse (*Pah* on chromosome 10 and *Rar-g* on chromosome 15; Ledley *et al.*, 1988; and this work). There are precedents for markers that are syntenic in one of these two rodent species and in man, but are not syntenic in the other rodent species (Levan *et al.*, 1991).

Finally, Table 2 indicates that some *RAR* genes are highly linked to *HOX* genes (at least in man and mouse, where the mapping data are available): *RARA* (*Rar-a*) and *HOX2* (*Hox-2*) genes colocalize on human chromosome 17, in the region q21–q22, and on mouse chromosome 11 band D (Mattei *et al.*, 1988a, and this work; Xu *et al.*, 1988; Buchberg *et al.*, 1989), whereas the *RARG* (*Rar-g*) and *HOX3* (*Hox-3*) genes map on human chromosome 12, in the region q12–q13, and on mouse chromosome 15 band F (this work and Rabin *et al.*, 1986). On the other hand, the *Rar-b* and the *Hox-1.6* genes are both on mouse chromosome 14, but they are not linked (bands A and E, respectively; this work and Sharpe *et al.*, 1988). It thus appears that duplications of *HOX* gene clusters, which probably generated multiple *HOX* genomic domains during mammal evolution (Hart *et al.*, 1987; Acampora *et al.*, 1989), also involved non-*Hox* genes like *RAR* genes. This synteny conservation of *HOX* and *RAR* genes is intriguing, taking into account the fact that retinoic acid is an inducer of *HOX* genes (Simeone *et al.*, 1990). On the basis of the data summarized in Table 2, it could be predicted that *HOX* genes reside on rat chromosome 10 (synteny with *RARA*) and chromosome 7 (synteny with *RARG*).

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