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

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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-α); 14, band A (Rar-β); and 15, band F (Rar-γ), 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). © 1991 Academic Press, Inc.

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; Petkovich 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., 1995a,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

Chromosomes spread preparations. In situ hybridizations were carried out on metaphase chromosomes spreads. These were obtained from phytohemagglutinin-stimulated human lymphocytes cultured for 72 h or from concanavalin A-stimulated mouse lymphocytes from a WMP/Pas inbred 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 × 10^6 dpm/μg. The probes used were the entire human cDNA insert, designated hRARγ (Krutz et al., 1989), and the three entire mouse cDNAs, α, β, and γ, designated mRARα, mRARβ, and mRARγ, respectively.
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 (78%) 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-α) 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 RARγ locus (Rar-γ) 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–A5 region of chromosome 14.

FIG. 4. Assignment of the mouse RARγ locus (Rar-γ) 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 could be identified in region E–F9 with a major hybridization peak in the band F.
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 EcoRI fragment from the pHK1 plasmid, containing the full-length human RARα cDNA (Giguere et al., 1987); the 1.4-kb Mael 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 3x SSC, 10x 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 (RARα and RARβ genes), these (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 26 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.

FIG. 5. Autoradiogram of a Southern blot of BamHI-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, LBR780.5 is positive for the 8.9- and 6.7-kb fragments only, LBB10 is positive for the three fragments, and LBB860 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).

FIG. 6. Autoradiogram of a Southern blot of HindIII-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.
concentrations were modified to 300 μg/ml and 0.5 ng/ml, respectively, and in the case of the \( \text{RAR}B \) 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 \( \text{in situ} \) 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% (75/158) 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 \( \text{RARA} \) gene. This cross-hybridization could be due to sequence homology between the \( \text{RARG} \) and \( \text{RARA} \) genes. Nevertheless, these results allow us to map the human \( \text{RARG} \) 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 \( \text{in situ} \) hybridization. In the case of the \( \text{Rar-a} \) 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 \( \text{RARa} \) gene to the 11D band of the mouse genome.

The \( \text{Rar-b} \) 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 \( \text{Rar-b} \) gene maps in the 14A band of the mouse genome.

Finally, in the case of the \( \text{Rar-g} \) 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 \( \text{RARG} \) 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 \( \text{Rar-a} \) gene (see above). The most probable localization of the mouse \( \text{Rar-g} \) gene is thus the F band of chromosome 15.

**The Three Rat RAR Genes**

The assignment of the three rat \( \text{RAR} \) 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. \( \text{BamHI} \) was used in the case of \( \text{RARA} \); the main rat-specific restriction fragments were detected at 8.9 and 6.7 kb (Fig. 5). \( \text{HindIII} \) was used in the case of \( \text{RARB} \); rat-specific restriction fragments were visible at 5.7 and 2.2 kb (Fig. 6). \( \text{BamHI} \) was also used in the case of \( \text{RARG} \); 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 \( \text{RAR} \) 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 \( \text{RARA} \) and rat chromosome 10, for \( \text{RARB} \) and rat chromosome 15, and for \( \text{RARG} \) 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

<table>
<thead>
<tr>
<th>Rat RAR genes</th>
<th>Rat chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Hybrids</td>
<td></td>
</tr>
<tr>
<td>LB20</td>
<td>N</td>
</tr>
<tr>
<td>LB150-1</td>
<td>+</td>
</tr>
<tr>
<td>LB161</td>
<td>-</td>
</tr>
<tr>
<td>LB210-I</td>
<td>-</td>
</tr>
<tr>
<td>LB261</td>
<td>+</td>
</tr>
<tr>
<td>LB330</td>
<td>N</td>
</tr>
<tr>
<td>LB330TG3</td>
<td>-</td>
</tr>
<tr>
<td>LB330TG6</td>
<td>N</td>
</tr>
<tr>
<td>LB510-6</td>
<td>+</td>
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<tr>
<td>LB600</td>
<td>-</td>
</tr>
<tr>
<td>LB603</td>
<td>-</td>
</tr>
<tr>
<td>LB780-6</td>
<td>-</td>
</tr>
<tr>
<td>LB780-8</td>
<td>N</td>
</tr>
<tr>
<td>LB810</td>
<td>+</td>
</tr>
<tr>
<td>LB860</td>
<td>-</td>
</tr>
<tr>
<td>LB1040TG1</td>
<td>-</td>
</tr>
<tr>
<td>LB1040TG3</td>
<td>N</td>
</tr>
<tr>
<td>LB1040TG5</td>
<td>+</td>
</tr>
</tbody>
</table>

Independent discordant clones:

<table>
<thead>
<tr>
<th>RAR</th>
<th>RAA</th>
<th>RAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

\*+ and - presence or absence of rat hybridization signal, respectively; (-), weak rat hybridization signal; N, not done.

\*+, 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.

Independent hybrid clones are clones derived from hybrid experiments. RAR are identified by independent numbers (nonindependent clones are, for instance, LB330TG3 and LB330TG6). When a RAR is 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 chromosomal genes 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

<table>
<thead>
<tr>
<th>Locus: Human symbol</th>
<th>Chromosome location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human (HSA)</td>
</tr>
<tr>
<td>Retinoic receptor α: RARA</td>
<td>17 q21.1</td>
</tr>
<tr>
<td>Homeobox-2: HOX2</td>
<td>17 q21–q22</td>
</tr>
<tr>
<td>Thyroid hormone receptor α: THRA1 (ERBA1)</td>
<td>17 q11.2–q12</td>
</tr>
<tr>
<td>AEV oncogene homolog 2: ERBB2 (rat neu)</td>
<td>17 q11.2–q12</td>
</tr>
<tr>
<td>Retinoic acid receptor β: RARB</td>
<td>3 p24</td>
</tr>
<tr>
<td>Thyroid receptor β: THRBB (ERBA2)</td>
<td>3 p24.1–p22</td>
</tr>
<tr>
<td>Retinoblastoma gene: RB1</td>
<td>13 q14.2</td>
</tr>
<tr>
<td>Retinoic acid receptor γ: RARG</td>
<td>12 q13</td>
</tr>
<tr>
<td>Homeobox-3: HOX3</td>
<td>12 q12–q13</td>
</tr>
<tr>
<td>Phenylalanine hydroxylase: PAH</td>
<td>12 q22–q24</td>
</tr>
<tr>
<td>MYC oncogene</td>
<td>8 q24</td>
</tr>
</tbody>
</table>

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 Sunre 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; Sziper et al., 1991). These two genes thus define a new conserved syntenic group in the two species. However, this syntenic group is not retained in man.

Like the RARB gene, the THRBB (ERBA2) maps on human chromosome 3 and on rat chromosome 15 (Dobrovic et al., 1988; Drabkin et al., 1988; Sziper et al., 1991). These two genes thus probably define a new syntenic 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–THRBB (ERBA2) syntenic 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 supports the hypothesis of a new syntenic group, comprising RARB and PAH, retained in man on chromosome 12 and in rat on chromosome 7 (for the assignments of PAH, see Lidsky et al., 1985; Fulchighnoni-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 Rap-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 Rap-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 mammalian 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 resides on rat chromosome 10 (synteny with RARA) and chromosome 7 (synteny with RARG).

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**REFERENCES**

1. Acampora, D., D’Esposito, M., Fadella, A., Pannese, M., Migliaccio, E., Morelli, F., Stornaiuolo, A., Negro, V.,


