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Compositional mapping of mouse chromosomes and identification of the gene-rich regions

S. Saccone, S. Caccio, P. Perani, L. Andreozzi, A. Rapisarda, S. Motta & G. Bernardi

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The mouse genome is a mosaic of isochores, consisting of long (> 300 kb), compositionally homogeneous DNA segments that can be divided into two GC-poor families, L1 and L2, representing 56% of the genome, and two GC-rich families, H1 and H2, representing 26% and 7% of the genome, respectively, the remaining 11% being formed by satellite and ribosomal DNAs. (GC is the molar fraction of guanine + cytosine in DNA.) The mouse genome differs from the human genome (which is representative of most mammalian genomes) because it shows a narrower compositional spectrum of isochores and it has a karyotype formed exclusively by acrocentric chromosomes. The chromosomal distribution of the four isochore families, as investigated here by *in situ* hybridization of single-copy sequences from compositional DNA fractions, has shown that G(iemsa) bands are essentially composed of GC-poor isochores, whereas R(everse) bands comprise three subsets of bands: R′ bands, containing GC-poor isochores and GC-rich isochores of the H1 family, and T and T′ bands, containing all H2 isochores (in addition to other isochores), the former containing a higher proportion of H2 isochores than the latter. Mouse T and T′ bands are generally syntenic with, and are compositionally related to, human T and T′ bands and have the highest gene concentrations. These findings indicate that the distribution of isochore families and genes in chromosomal bands is basically similar in mouse and in human genomes, in spite of their remarkable differences and their extremely large phylogenetic distance.

Key words: chromosomal bands, *in situ* hybridization, isochores, human–mouse synteny

Introduction

Vertebrate genomes are mosaics of isochores, which are (> 300 kb), compositionally homogeneous DNA segments that can be divided into a small number of families that have different GC levels. (GC is the molar fraction of guanine + cytosine in DNA.) In the case of the human genome, which is the most extensively studied (see Bernardi, 1995, for a recent review), five isochore families have been identified: two GC-poor families, L1 and L2, representing 62% of the genome, and three GC-rich isochore families, H1, H2 and H3, accounting for 21%, 9% and 3–4% of the genome, respectively, the remaining 4% being formed by satellite and ribosomal DNAs. Isochore families differ not only in GC levels but also in gene concentration, which increases with increasing GC levels of isochores. Indeed, the human GC-richest isochore family, H3, has a gene concentration about 20 times higher than that of the GC-poor isochore families (Mouchiroud *et al.* 1991; Zoubak *et al.* 1996).

The correlation between isochores and chromosomal bands has been investigated by 'chromosomal' compositional mapping, i.e. by *in situ* hybridization of compositional DNA fractions on human metaphase chromosomes (Saccone *et al.* 1992, 1993, 1996) and, at a higher resolution, by 'molecular' compositional mapping of specific chromosome arms or regions (Gardiner *et al.* 1990, Bettecken *et al.* 1992, Pilia *et al.* 1993, De Sario *et al.* 1996). Briefly, both 'chromosomal' and 'molecular' compositional mapping have shown that, in the human genome, G(iemsa) bands are essentially composed of GC-poor, gene-poor isochores of the L1 and L2 families, whereas R(everse) bands are composed, on average, of both GC-poor and GC-rich, gene-rich, isochore families. Of great interest was the finding that the GC-richest and gene-richest isochore family H3 hybridized strongly on 28 R bands (Saccone *et al.* 1992), which largely coincide with T(elomeric) bands (the most heat denaturation-resistant subset of R bands; Dutrillaux 1973) and hybridized weakly on 31 T′ bands, whereas the remaining 140 R bands (at a 400-band resolution) did not contain H3 isochores and seemed to be characterized by GC levels that were, on average, very close to those of the G bands (Saccone *et al.* 1996).

The isochore organization of the human genome outlined above is shared by the majority of mammalian orders, as demonstrated by compositional analysis of DNA (Sabeur *et al.* 1993) and coding sequences (Mou-

S. Saccone, L. Andreozzi, A. Rapisarda and S. Motta are at the Dipartimento di Biologia Animale, Università di Catania, Via Androne 81, 95124 Catania, Italy. S Cacciò, P. Perani and G. Bernardi (corresponding author) are at the Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2, Place Jussieu, 75005 Paris, France. Tel: (+33) 1 44 27 81 72; Fax: (+33) 1 44 27 79 77; Email: Bernardi@Citi2.FR. S. Cacciò is presently at the Laboratorio di Parassitologia, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Roma, Italy. P. Perani is presently at the Unité INSERM 450, 29, Rue Wilhem, 75016 Paris, France.

chiroud & Bernardi 1993), and by cross-specific hybridization experiments using human H3 isochores as the probe (Cacciò *et al.* 1994). The human genome therefore exemplifies what has been called the 'general mammalian pattern' (Sabeur *et al.* 1993).

A very interesting deviation from this pattern is represented by species belongining to the Myomorpha suborder of Rodentia that comprises Murids, such as mouse and rat. Indeed, the genomes of these species display a compositionally narrower distribution of DNA fragments (Salinas *et al.* 1986, Zerial *et al.* 1986, Bernardi *et al.* 1988, Mouchiroud *et al.* 1988). The scarcity of very GC-rich isochores also accounts for the reduced number of CpG islands present in the mouse genome (Aïssani & Bernardi 1991a & b, Antequera & Bird 1993, Matsuo *et al.* 1993), as those sequences are mainly found in the GC-richest isochores. Another difference between the mouse and the human genome is that all chromosomes of the former are acrocentric.

The remarkable differences between the mouse and the human genomes and the crucial importance of the mouse genome as a model genome have prompted us to investigate the distribution of isochore families on mouse metaphase chromosomes using *in situ* hybridization of compositional mouse DNA fractions.

Materials and methods

DNA fractionation

High-molecular-weight DNA (50–100 kb) was isolated from mouse liver and fractionated on a Cs_2SO_4/BAMD gradient as described previously (Thiery *et al.* 1976, Cuny *et al.* 1981, Salinas *et al.* 1986). BAMD is 3,6-bis (acetatomercurimethyl)-1,4 dioxane. A nucleotide–BAMD molar ratio, rf, of 0.14 was used. The compositional DNA fractions obtained were analysed by CsCl analytical untracentrifugation and HPLC (high-performance liquid chromatography).

Chromosome preparation and *in situ* hybridization

Mouse metaphase chromosomes were obtained from disrupted spleen cells that were grown in RPMI-1640 medium supplemented with 10% fetal calf serum and 3% PHA (phytohaemagglutinin). Cells were incubated at 37°C for 72 h, then colcemid (0.1 μg/ml) was added 45 min before they were harvested with hypotonic solution (75 mM KCl) for 15 min at 37°C. Conventional methanol–acetic acid fixation and slide preparation were used. Conditions for chromosome pretreatment and denaturation were as described for human chromosomes (Saccone *et al.* 1992). Samples (100–200 ng) of each biotinylated DNA fraction were co-precipitated with a 100-fold excess of carrier yeast t-RNA and a 500-fold excess of sheared total genomic mouse DNA added as a competitor. Each nucleic acid solution was resuspended in 10 μl of 50% formamide, 2 × SSC (standard saline citrate; 0.15 M NaCl, 0.015 M sodium citrate), 10% dextran sulphate, 50 mM sodium phosphate, pH 7.0, and denatured at 80°C for 10 min. A preannealing step was performed by incubating the hybridization mixtures at 37°C for 30–60 min. Hybridization, washings and signal detection were performed as described previously (Saccone *et al.* 1992). Under the conditions used, only single-copy DNA sequences were allowed to hybridize. As expected, centromeres were always depleted of signals, indicating that the contribution of the major and minor satellite DNAs, present in fractions 1–5, was effectively suppressed.

Results

DNA fractionation

Table 1 shows the relative amount and the GC level of each compositional mouse DNA fraction as well as the isochore families represented in each fraction. The estimates of the isochore families were based on a comparison with previous data (Salinas *et al.* 1986) that had shown that GC-poor isochores of the L1 and L2 families represent about 63% of the mouse genome (including about 7% of major satellite DNA), whereas GC-rich isochores of the H1 and H2 families represent 26% and 7% of the genome, respectively, the remaining 4% being represented by ribosomal and minor satellite DNAs.

In situ hybridization

Figure 1 shows the hybridization patterns obtained with the mouse compositional DNA fractions (see Table 1). Hybridization with fraction 1 (Figure 1A), corresponding to the DNA pellet from the Cs_2CO_4/BAMD centrifugation (namely the GC-poorest DNA), and with pooled fractions 2–6 (Figure 1B) stained all chromosomes, but not uniformly. Hybridization signals obtained with fraction 7 (Figure 1C) were distributed over R bands and a number of G bands, whereas those due to DNA from fraction 8 gave a signal distribution that was closer to R banding (Figure 1D). An obvious R banding pattern was obtained with the H1 + H2 isochores present in fraction 9 (Figure 1E), whereas signals from pooled

Table 1. Mouse compositional DNA fractions used for *in situ* hybridization

Fraction number[a]	DNA (%)	GC (%)[b]	Isochore family[c]
1			L1 + major satellite DNA
2			L1
3			L2
4			L2
5			L2
6			L2 + *H1*
7			*L2* + H1
8			H1
9			H1 + *H2*
10			H2
11			H2 + ribosomal DNA

[a]Fraction 1 is the pellet, which also contains the major satellite DNA (Salinas *et al.* 1986). [b]Determined by HPLC. [c]Estimates are based on comparisons of relative amounts of DNA and GC levels with previous work (Salinas *et al.* 1986). Italics indicate the minority isochore family present in the fractions.

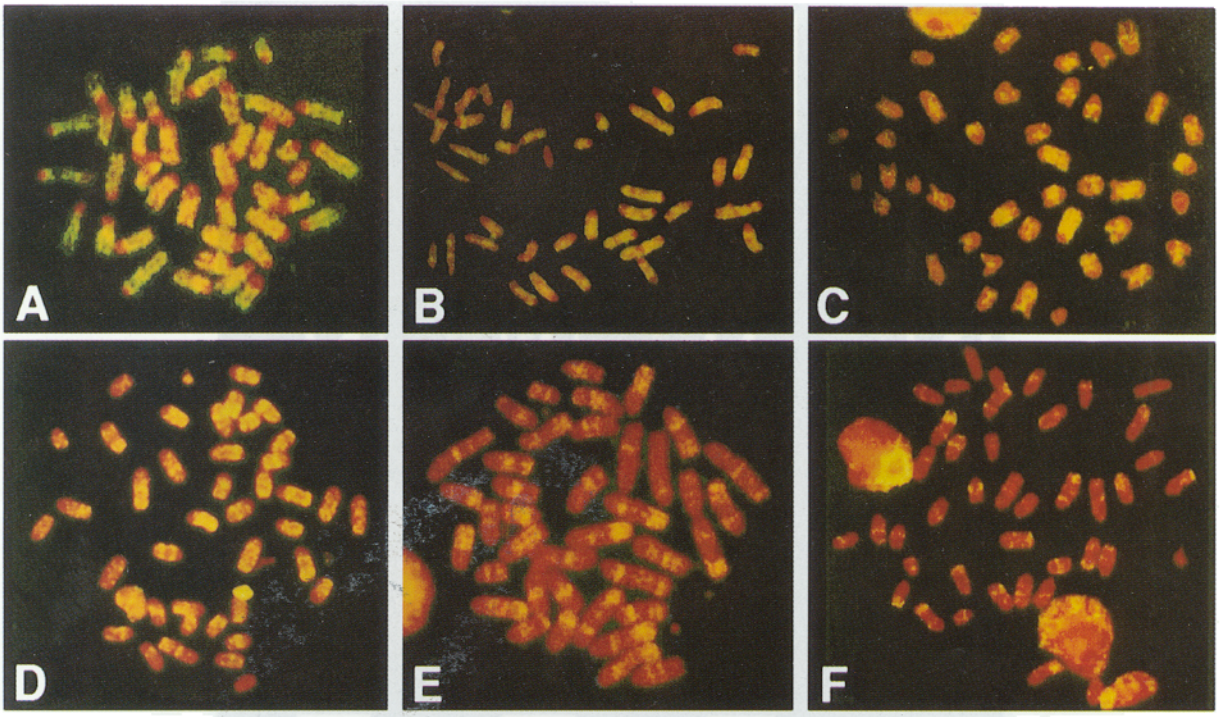

Figure 1. ***In situ*** hybridization of mouse DNA fractions (see Table 1) on metaphase chromosomes: **A** fraction 1, derived from isochore family L1; **B** pool of fractions 2–6, derived from isochore families L1 + L2 with a minor contribution from H1 isochores; **C** fraction 7, derived from isochore family H1 with a minor contribution from L2 isochores; **D** fraction 8, derived from the isochore family H1; **E** fraction 9, derived from the isochore family H1 with a minor contribution from H2 isochores; **F** pool of fractions 10 and 11, derived from the isochore family H2. Biotinylated probe was detected with avidin–FITC, and chromosomes were stained with propidium iodide.

fractions 10 and 11 containing only H2 isochores were distributed on only a subset of R bands (Figure 1F). Figure 1 clearly indicates that, when the GC-poor isochores were hybridized (Figure 1A–C), it was not possible to construct a karyotype. In contrast, when the GC-rich isochores were hybridized (Figure 1D–F), the hybridization pattern was more clearly defined and individual chromosomes were easily identified.

It should be noted that the small unlabelled chromosome that is particularly evident in Figure 1B–D is the Y chromosome (see also Figure 2).

Chromosomal distribution of the GC-richest isochores

The analysis of 20 metaphases hybridized with DNA from H2 isochores showed that the GC-richest isochores were present in 30 R bands, in which hybridization signals were present in all metaphases investigated, and in 13 additional R bands, in which hybridization signals were variable both in intensity and frequency. On this basis, as well as on that of human–mouse comparative compositional mapping (see below), in order to follow the nomenclature developed for human chromosomal bands, we called the first set T (or $H2^+$) bands and the second T′ (or $H2^*$) bands. In other words, we interpreted the difference between T and T′ bands as being due to the different proportion of H2 isochores present in those bands, as previously observed in the human genome (Saccone *et al.* 1996). While Figure 1F shows an example of an H2 DNA hybridization on all T bands but on only some T′ bands, Figure 2 corresponds to a metaphase that exhibits a hybridization on all T and T′ bands. Figure 2 shows that: (1) all the G bands and a number of the R bands were depleted of hybridization signals; (2) hybridization signals were evident in many telomeric regions, and only some autosomes (chromosomes 3, 9, 10, 13, 14 and 17) showed scarce or no signals near the telomeres; and (3) the sex chromosomes showed weak signals over their entire length and represented the only chromosomes with no detectable hybridization.

Chromosomal distribution of repeated sequences

Previous results (Soriano *et al.* 1983) have shown that members of the B1 family of interspersed repeats in the mouse are most frequent in GC-rich isochores. As expected, the results obtained with mouse GC-rich isochores (Figure 2) are consistent with the *in situ* hybridization data obtained with the mouse short-

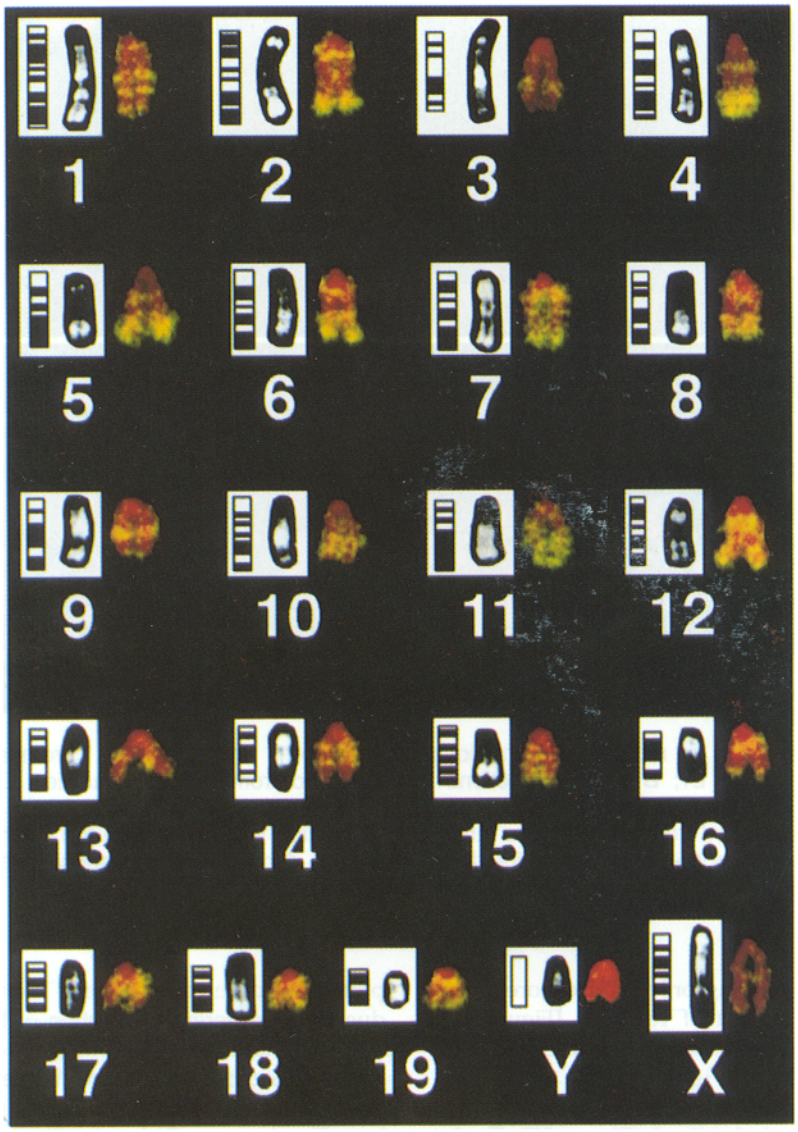

Figure 2. Mouse haploid male karyotype constructed from a metaphase hybridized with mouse H2 DNA and showing all T and T′ bands. Biotinylated probe was detected with avidin–FITC and chromosomes were stained with propidium iodide. Right, hybridization pattern; centre, the R banded chromosome (from Somssich *et al.* 1981); left, the idiogram of R-banded chromosomes.

interspersed repeated sequences (SINEs B1 and B2) that have been localized in the R bands of mouse chromosomes (Boyle *et al.* 1990). More precisely, the H2-positive bands mostly correspond to the stronger bands obtained by the hybridization with the SINEs (as evaluated from Figure 2 of Boyle *et al.* 1990), as well as to the early-replicating bands (Somssich *et al.* 1981; see also Figure 2) with the exception of a few chromosomal bands that are more intense in H2 banding. The same correspondence was observed by comparing the *in situ* hybridizations of H3 isochores (Saccone *et al.* 1992) and *Alu* sequences (Manuelidis & Ward 1984, Korenberg & Rykowski 1988) in human chromosomes.

Human–mouse comparative compositional mapping

The hybridization obtained here with the mouse H2 isochore family on mouse chromosomes (Figure 2) was compared with the hybridization obtained with the human H3 isochore family on human chromosomes (Saccone *et al.* 1996). Taking into account the human–mouse homology relationships based on genetic and physical maps (Copeland *et al.* 1993, Lyon & Kirby, 1994, DeBry & Seldin 1996), this allows the identification of the regions of the mouse chromosomes that correspond to the GC-richest, gene-richest human chromosomal bands. The results shown in Figure 3 indicate

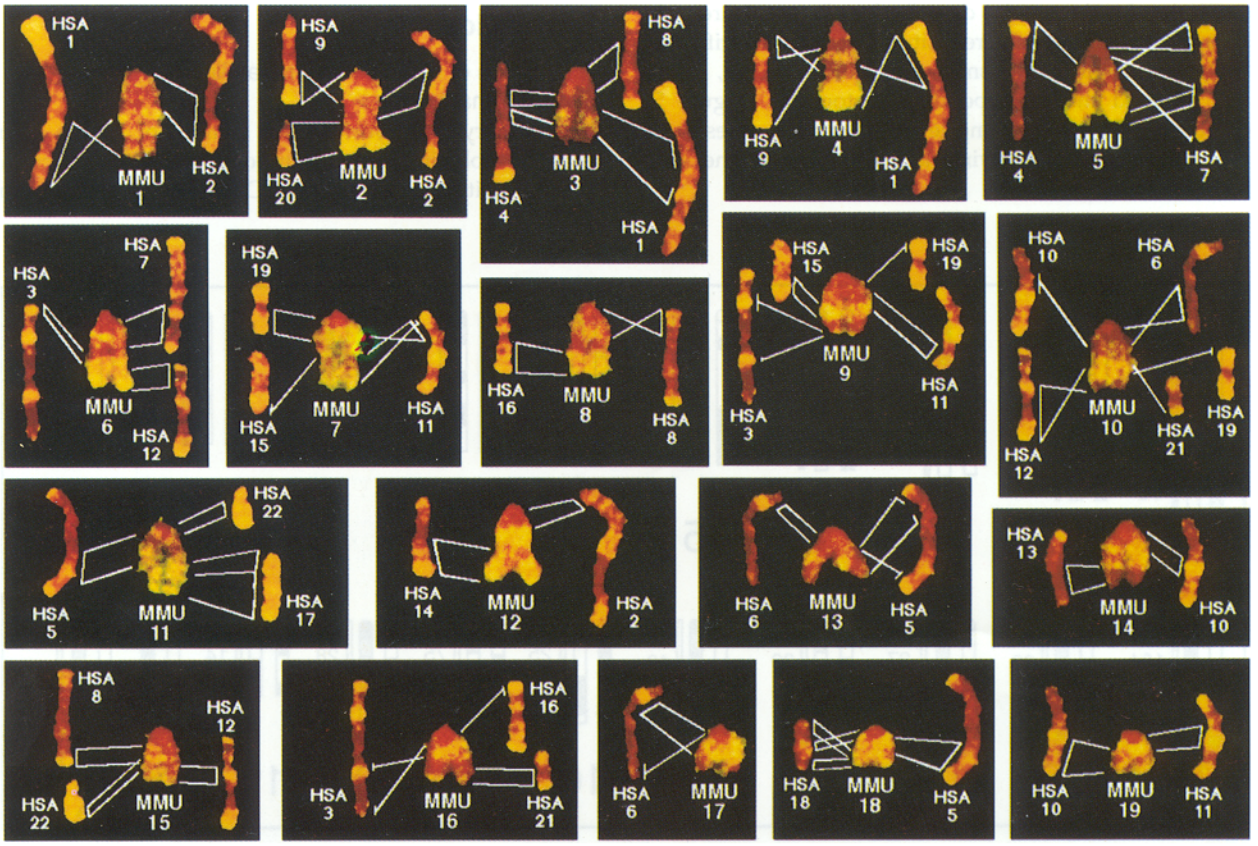

Figure 3. Identification of the syntenic regions between mouse and human chromosomes. MMU, *Mus musculus*; HSA, *Homo sapiens*. For clarity, chromosome HSA4 in the syntenic panel with MMU3 is shown in an inverted position. Human chromosomes with small syntenic regions or not involving T or T′ bands are not indicated. Human chromosomes are from Figure 2 of Saccone *et al.* (1996).

that many regions of the mouse and human chromosomes are compositionally conserved in spite of the remarkable differences between the two genomes. For example, the entire human chromosome 20 is syntenic with the distal part of the mouse chromosome 2, and the syntenic subregions present the same compositional pattern. The same compositional correspondence is clearly visible in the human chromosome 17, which is syntenic with the distal part of the mouse chromosome 11, and also in the p and q arms of the human chromosome 1, which are syntenic with the regions of the mouse chromosomes 4 and 1 respectively. Many other mouse $H2^+$ bands share the compositional pattern of the syntenic human chromosomal $H3^+$ bands, as shown in Figure 3. It should be noted that all mouse chromosomes are represented in Figure 3. Many of these clearly show a compositional correlation with the human chromosomes.

Gene distribution

Figure 4 shows a scheme of T and G bands in the mouse karotype, the regions considered here to be 'telomeric' and the number of loci (estimated from DeBry & Seldin 1996) present in the 'telomeric' and 'non-telomeric' regions.

Figure 5 indicates that, in most chromosomes, 'telomeric' regions exhibit higher gene concentrations than 'non-telomeric' regions. Most of the exceptions are chromosomes that do not show 'telomeric' T or T′ bands (namely chromosomes 3, 10, 13, 14, and 17) and that are also characterized by low gene concentrations. It should be noted that chromosome 9 (like the X chromosome) does not show a T or T′ band in the 'telomeric' region, where it exhibits a high gene concentration, and that chromosome 11 shows a higher gene concentration in the 'non-telomeric' region than in the 'telomeric' regions.

The genes listed were derived from the 1416 loci described by DeBry & Seldin (1996). More specifically, we first considered those loci that were localized both physically and genetically (Lyon & Kirby 1994); we then assumed that all loci that were localized only genetically between two loci previously identified were physically located in the chromosomal region defined by them.

Obviously, the results described in Figure 4 are only an approximation of the real situation. More specifically, the genes listed on the smaller chromosome (16, 17, 18 and 19) and the corresponding histograms (Figure 5) could be affected by some level of error as a result of the difficulty of identifying loci anchored to the subterminal bands.

Discussion

Distribution of mouse isochore families on chromosomal bands

In summary, the hybridization pattern of mouse chromosomes obtained with compositional DNA fractions changes with the increasing GC level of the latter from

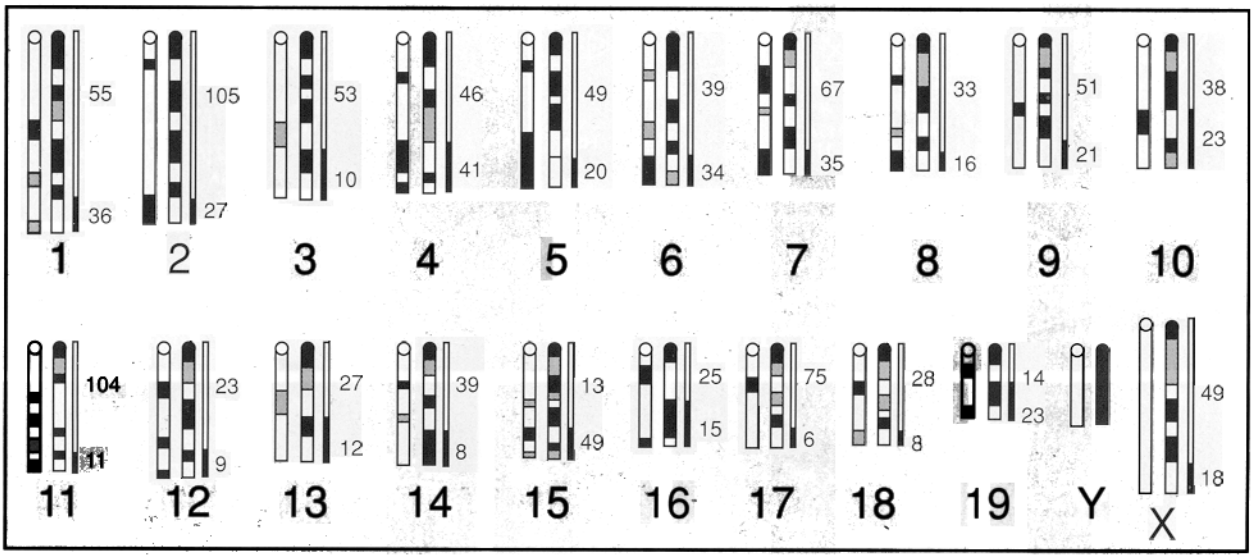

Figure 4. Gene distribution and bands on mouse chromosomes. Left, H2-positive bands as estimated by H2 isochore hybridization (black and grey bands correspond to T and T′ bands respectively); centre, G bands (modified from Lyon & Kirby 1994; only the major bands are shown); right, bars indicating the 'telomeric' and the 'non-telomeric' regions, with the number of loci localized (from DeBry & Seldin 1990, taking into account the relationship between the genetic and physical maps of Lyon & Kirby 1994). Loci not identified as genes were not considered. When the identification of the telomeric region loci was ambiguous, because of the limited information available (namely for chromosomes 3, 4, 6, 10, 12, 13, 15 and 19), subtelomeric bands were included in the 'telomeric' regions.

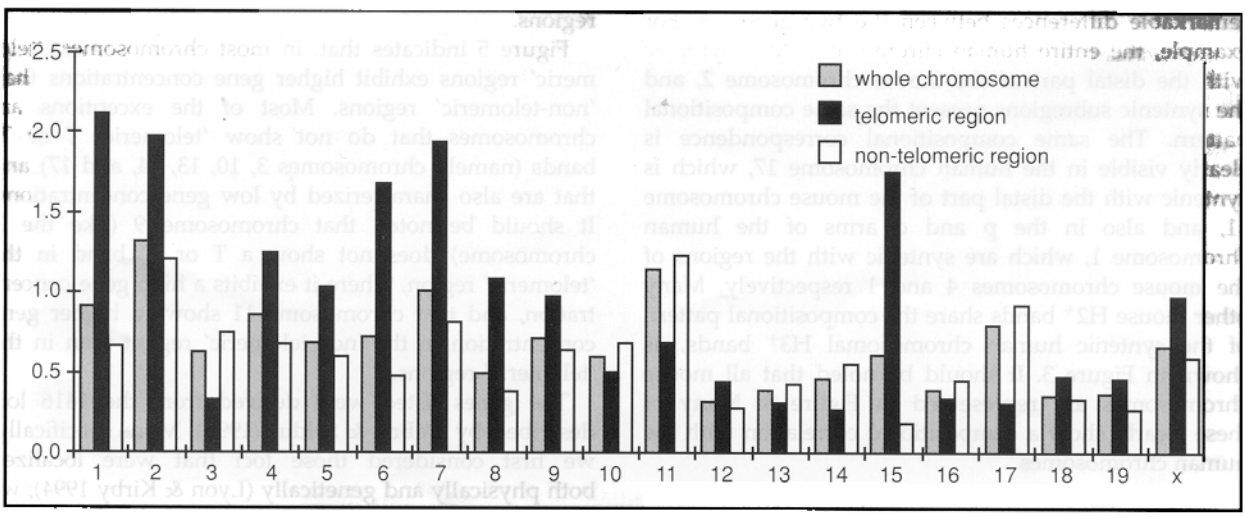

Figure 5. Histogram showing the gene concentration (gene number divided by the length of chromosomes or chromosome regions in arbitrary units) for entire chromosomes (cross-hatched bars), 'telomeric regions' (black bars) and 'non-telomeric' regions (white bars). The Y chromosome is not shown in this figure.

a very diffuse staining over all chromosomes to an increasingly specific signal distribution over R bands and finally to an even more specific signal distribution over a subset of R bands. More specifically, as far as GC-poor isochores are concerned, the data clearly show their distribution over all G bands and most R bands of the mouse karyotype (Figure 1A and B). Hybridization with DNA derived from the mouse H1 isochore family produced a pattern largely corresponding to an R banding (Figure 1C–E). Finally, hybridization with mouse H2 isochores is restricted to a subset of R bands (Figure 1F and Figure 2) that were called T (or $H2^+$) and T′ (or $H2^*$) bands, the former containing a higher concentration of H2 isochores than the latter. In the case of hybridization with GC-rich isochores, chromosomes could be easily identified.

The ratio of DNA amounts in G and R bands of mouse chromosomes (as derived from published idiograms) is about 55% to 45%, respectively if one pools the centromeric regions with G bands and neglects possible different degrees of DNA compaction in G and R bands (Bernardi 1989). As already mentioned, the isochore families L1 + L2, H1 and H2 represent 63% (including 7% satellite DNA), 26% and 7% of the mouse genome respectively. If we assume, as a first approximation, that G bands are composed only of L1 and L2 isochores, then R bands are composed of the totality of H2 and H1 isochores (25% + 7% of the genome), the remaining 13% of R band DNA being represented by L1 and L2 isochores. Figure 1C and D indicates, however, that H1 isochores are present in G bands, even if their amounts can not be quantified. Obviously, the presence of any H1 isochores in G bands would be compensated by an increased amount of L1 and L2 isochores in R bands. For example, if H1 isochores were present at a level of 5% in G bands, this would increase the percentage of L isochores in R bands from 13% to 18%.

Figure 1F and Figure 2 show that hybridization with the GC-richest isochores identifies two subsets of R bands not previously described, called here T and T′ bands, that can be distinguished from other R bands (called here R′ bands) by the presence of H2 isochores. On the basis of published karyotypes, and of Figure 4, we estimate that T, T′ and R′ bands account for 16%, 5% and 24% of mouse DNA respectively.

Syntenic regions between mouse and human chromosomes

Several lines of evidence indicate that mouse $H2^+$ bands are the equivalent of human $H3^+$ bands: (1) single-copy DNA sequences of the GC-richest isochores from all warm-blooded vertebrates (mammals and birds) cross-hybridize with each other (Cacciò *et al.* 1994); (2) a comparison of orthologous coding sequences indicate that, as far as GC levels are concerned, compositional gene ranking is largely similar in the human and mouse genomes (and, more generally, in vertebrate genomes; see Bernardi 1995), in spite of the compositional differences between these genomes; (3) CpG islands are concentrated in GC-rich isochores in both the human and mouse genomes (Aïssani & Bernardi 1991a & b).

A detailed comparison is not easy in that our previous results on the human chromosomes concerned a 400-band pattern, whereas the mouse results were obtained at a resolution of only 110–120 bands. However, it can be stated (1) that the majority of human T bands correspond to mouse T bands, the rest corresponding to T′ bands, to complex bands (corresponding to more than one human band and comprising more than one human T or T′ band) or to bands not hybridizing H2 isochores; (2) that human T′ bands correspond, in roughly the same proportions, to mouse T bands, T′ bands, complex bands and to bands not hybridizing H2 isochores; and (3) that the 'telomeric' regions of the mouse chromosomes contain a high gene concentration compared with the 'non-telomeric' regions. These data indicate that, as in the case of the human genome (Zoubak *et al.* 1996), the GC-richest isochores are the gene-richest regions of the mouse genome. In human chromosomes, these regions are characterized by important properties, such as an open chromatin structure, and high recombinational and transcription activities that are likely to be conserved in the corresponding regions of mouse chromosomes (as well as in those of chromosomes from other mammalian species).

Two concluding remarks are appropriate at this point. The first one is that the hybridization of GC-richest isochores is a novel approach that can be used next to existing methods to compare different mammalian karyotypes and to investigate the evolution of T and T′ bands. So far, comparative chromosomal banding and synteny studies have been performed using human chromosome-specific DNA libraries as probes to hybridize on chromosomes of related mammalian species. The second remark concerns evidence accumulating in favour of the suggestion (Mouchiroud *et al.* 1988) that the isochore pattern of Myomorpha is not derived from the general mammalian pattern but is a primitive one. Indeed, Myomorpha were among the first Eutherians to branch off, perhaps 120 million years ago (Janke *et al.* 1997). Under these circumstances, the human–mouse synteny and the homology of human–mouse T bands stress the very strong conservation of band patterns of isochore and gene distribution over the chromosomes of mammals.

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