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Genetics

Correlations between isochores and chromosomal bands in the human genome

(*in situ* hybridization/chromosome banding/compositional map)

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ABSTRACT **The human genome is made up of long DNA segments, the isochores, which are compositionally homogeneous and can be subdivided into a small number of families characterized by different G+C levels. Chromosome *in situ* suppression hybridization (in which excess unlabeled human DNA is added to suppress hybridization of repeated sequences present in the probe, enabling enhanced observation of single-copy sequences) of DNA fractions characterized by an increasing G+C level was carried out to determine the distribution of "single-copy" sequences corresponding to isochore families L1 + L2, H1, H2, and H3 on metaphase chromosomes. This produced a banding pattern progressing from a relatively diffuse staining to an R-banding, to a T-banding. More specifically, our results showed that (*i*) T-bands are formed by the G+C-richest isochores of the H3 family and by part of the G+C-rich isochores of the H1 and H2 families (with a predominance of the latter); (*ii*) R′-bands (namely, R-bands exclusive of T-bands) are formed to almost equal extents by G+C-rich isochores of the H1 families (with a minor contribution of the H2 and H3 families) and by G+C-poor isochores of the L1 + L2 families; (*iii*) G-bands essentially consist of G+C-poor isochores from the L1 + L2 families, with a minor contribution of isochores from the H1 family. These results not only clarify the correlations between DNA base composition and chromosomal bands but also provide information on the distribution of genes in chromosomes, gene concentration increasing with the G+C levels of isochores.**

Vertebrate genomes (like the genomes of plants and other eukaryotes) are made up of long DNA segments (several hundred kilobases in size) called the isochores, which are compositionally homogeneous and can be subdivided into a small number of families characterized by different G+C levels (1, 2). In the human genome, which is representative of the genomes from the majority of mammalian Orders (3), it has been estimated (refs. 4 and 5; see *Results* for more details) that G+C-poor isochore families L1 ($\rho = 1.698$) and L2 ($\rho = 1.700$) make up about 62% of the genome, G+C-rich isochore families H1 ($\rho = 1.704$) and H2 ($\rho = 1.708$) represent together about 31% of the genome, and the G+C-richest isochore family H3 ($\rho = 1.712$) corresponds to 3–5% of the genome; the remaining part of the genome is formed by satellite DNAs and minor components (like ribosomal DNA), which can also be considered isochore families because of their compositional homogeneity.

Human metaphase chromosomes can be resolved by different experimental approaches into three main classes of bands, centromeric (C), Giemsa (G) and reverse (R) bands. A subset of the latter, the T-bands, are the most resistant to heat denaturation and are mainly localized at a number of telomeres, although several of them are internal (ref. 6; T-bands essentially coincide with chromomycin A3-positive, 4,6-diamino-2-phenylindole-negative bands; see ref. 7). While C-bands are known to correspond to satellite DNAs, a number of results (summarized in ref. 8) point to the fact that the DNA of G- and R-bands largely correspond to G+C-poor and G+C-rich isochores. However, it was stressed (8) (*i*) that this general correlation between isochores and chromosomal bands could only be considered as an approximation of the actual situation; (*ii*) that G-bands are remarkably homogeneous in DNA composition because they are made up of G+C-poor isochores that differ very little from each other in composition; and (*iii*) that R-bands are heterogeneous, since the corresponding G+C-rich isochores encompass a wide G+C range and since R-bands contain G+C-poor isochores corresponding to "thin" G-bands (as revealed by high-resolution banding; see ref. 9). In fact, the compositional heterogeneity of R-bands has been indicated by the fact that R- and G-bands are in a 1:1 ratio while G+C-rich and G+C-poor isochores are in a 1:2 ratio (8, 10).

A way to improve our understanding of the correlation between isochores of different G+C levels and chromosomal bands is compositional mapping, which consists in determining the base composition in DNA regions defined by a physical map (8, 10) or in chromosomal regions (11).

The first compositional map at the DNA level, that of the long arm of human chromosome 21, revealed that sequences located in G-bands corresponded to G+C-poor isochores, sequences located in R-bands corresponded to both G+C-poor and G+C-rich isochores, and sequences located in the telomeric R-band corresponded to G+C-rich and very G+C-rich isochores (10). This telomeric R-band is, in fact, a T-band.

The latter finding led to the proposal (8, 10) that the G+C-richest isochore family, H3, is located in T-bands. In agreement with this proposal, probes for sequences located in telomeric T-bands were shown to hybridize on H3 isochores, whereas the telomeric repeat, common to all chromosomes, hybridized not only on H3 but also on H1 and H2 isochores, indicating that the terminal 100 kb or so of telomeric regions are G+C-rich or very G+C-rich (12). Moreover, genes located in T-bands have a much higher G+C level in third codon positions (as expected of genes largely located in the G+C-richest isochores; see refs. 1, 8, 13, and 14) than do genes located in either G- or R-bands, the latter being only slightly richer in G+C than the former (12, 15).

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Abbreviations: T-bands, telomeric bands; R-bands, reverse bands; R′-bands, R-bands exclusive of T-bands; G-bands, Giemsa bands.

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The first compositional map at the cytogenetic level was obtained by chromosome *in situ* suppression hybridization (in which excess unlabeled total human DNA is added to suppress hybridization of repeated sequences present in the probe, enabling enhanced observation of single-copy sequences) of a human DNA fraction corresponding to the isochores of the H3 family. It showed that single-copy sequences from the G+C-richest human isochores are indeed localized in all T-bands of metaphase chromosomes (11) as previously proposed (8, 10). The rationale for localizing first the G+C-richest isochores of the H3 family was that this family is not only characterized by the highest gene concentration (13) and the highest CpG islands concentration (16, 17) but also by the highest transcriptional and recombinational activity (18) and by the abundance of a distinct, open chromatin structure (16, 17, 19).

In the present work, we have extended our investigations on the chromosomal localization of H3 isochores to the other two G+C-rich isochore families, H1 and H2, and to the G+C-poor isochore families, L1 and L2. The results obtained clarify the relationships between isochores and chromosomal bands. Moreover, because of the correlation between gene concentration and G+C levels of isochores (13), they shed light on the chromosomal distribution of genes.

MATERIALS AND METHODS

DNA Preparation. Human DNA (50-kb average fragment size as assessed by gel electrophoresis with phage λ DNA as a size marker) was extracted from placenta as described (5) and fractionated according to base composition on a preparative Cs_2SO_4/3,6-bis(acetatomercurimethyl)-1,4-dioxane density gradient at a ligand/nucleotide molar ratio of 0.14 (5). After dialysis, each fraction was analyzed for its buoyant density profile by CsCl density gradient centrifugation (20).

***In Situ* Hybridization.** Metaphase spreads were treated with RNase A at 100 μg/ml in 2× standard saline citrate (SSC; 1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) for 1 hr at 37°C, washed four times (5 min each) with 2× SSC, and then incubated with pepsin at 0.1 mg/ml in 0.01 M HCl for 10 min at 37°C. After two washes in phosphate-buffered saline (PBS) at pH 7.5, samples were fixed in 1% formaldehyde (freshly prepared in PBS containing 50 mM $MgCl_2$) for 10 min and then dried with a series of ethanol washes. The air-dried preparations were denatured in 70% formamide/2× SSC at 80°C for 3 min.

For each hybridization, 100 ng of each single human DNA fraction, labeled by nick-translation with biotin-16-dUTP (16 refers to a 16-atom spacer), was precipitated with a 50× excess of salmon sperm DNA and yeast tRNA (as carriers) and a 50× excess of Cot 1 human DNA (enriched for repetitive sequences) from GIBCO/BRL (as competitor DNA of repetitive sequences present in the probes). The nucleic acid mixture was resuspended in 10 μl of 50% formamide/2× SSC/10% dextran sulfate/50 mM sodium phosphate, pH 7.0, and denatured at 75–80°C for 5–7 min. A prehybridization step was then performed by incubating the sample at 37°C for 30 min, and hybridization was carried out overnight at 37°C in a humidified box.

Posthybridization washes were in 50% formamide/2× SSC, pH 7.0, at 42°C and in 0.1× SSC at 60°C. The blocking step was performed by incubation of the slides in 4× SSC/5% nonfat dry milk, and hybridization was detected with avidin conjugated to fluorescein isothiocyanate. Signals were obtained in a single step with biotinylated goat anti-avidin. Chromosomes were counterstained with propidium iodide.

RESULTS

DNA Fractionation Results. Fig. 1 displays the CsCl profiles of human DNA fractions obtained by preparative ultracentrifugation in Cs_2SO_4/3,6-bis(acetatomercurimethyl)-1,4-dioxane. DNA aliquots from fractions 1–9 were used as probes in the hybridization experiments to be described.

The pellet and fractions 1–4 correspond to isochores L1 + L2, since these fractions cover the buoyant density range of these isochore families and correspond in relative amount (62.7% of the genome) to them. Indeed, the amount of L1 + L2 isochores was estimated to be 65% by combined Cs_2SO_4/Ag^+ and CsCl analysis (20) and 62% by preparative fractionation in Cs_2SO_4/Ag^+ (4).

Fractions 5–7 and fractions 8 and 9 essentially correspond to isochores H1 and H2, respectively, since these two sets of fractions correspond in buoyant density range and relative amounts to previous estimates. Indeed, fractions 5–7 represent 22.6% of the genome, a value comparable to previous estimates of 18% (20) and 22% (4) for H1 isochores. Fractions 8 and 9 also are close in buoyant density and in relative amounts (8.7%) to previous estimates, 11% (20) and 9% (4) for H2 isochores.

Fractions 10 and 11 correspond to H3 isochores. Indeed, they are comparable in buoyant density and relative amount (4.5%) to values previously reported (see figure 1 *a* and *b* of ref. 5). Fraction 10 was the fraction used in previous work (11) on the localization of isochore family H3 in human chromosomes. Fraction 11 contained, in addition, a G+C-poor satellite DNA (ρ_o = 1.700 g/cm^3).

It should be noted that isochore families overlap with each other, especially at the relatively low molecular size (about 50

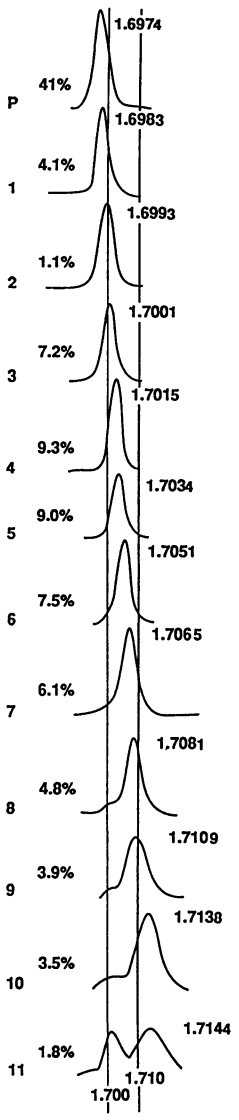

FIG. 1. Analytical CsCl profiles of human DNA fractions. The modal buoyant density and the relative amount of DNA of each fraction are indicated. P, pellet.

kb) of the DNA preparation fractionated in this work. This overlap decreases when moving from the abundant G+C-poor isochores, which are very close to each other in composition, to the more scarce G+C-rich isochores, which are more distant in composition. Obviously, isochore families can only occasionally coincide with preparative DNA fractions. In the case of Fig. 1, the border of H1 and of H2 isochores should be very slightly moved into fraction 7. Likewise, fraction 9 showed a broader CsCl profile compared with other fractions and certainly contained some of the H3 isochores that formed fraction 10 (see above). These considerations led to estimated amounts (based on human DNA exclusive of satellite and ribosomal DNAs) of 62%, 22%, 10%, and 5% for isochore families L1 + L2, H1, H2, and H3.

***In Situ* Hybridization Results.** *In situ* hybridization of DNA from the pooled fractions 1–4 stained whole chromosomes, but not uniformly; there was, in fact, a hint of G-banding. A widespread staining was expected because the L1 + L2 isochores not only correspond to almost all of the DNA present in G-bands, but also are well represented (see below) in R′-bands (as we will call the R-bands, if a subset of them, the T-bands, are neglected), while they are almost absent from T-bands (Fig. 2*A*).

Hybridization of individual fractions 5–9 showed a banding pattern that progressively changed from R to T. Fraction 5 produced an unclear R-banding pattern, some bands lacking sharpness and rather corresponding to clusters of dots (Fig. 2*B*). Fraction 6 produced a much better R-band pattern, with sharp bands and easily recognizable chromosomes (Fig. 2*C*). Fraction 7 showed the most complete and sharpest R-band pattern (Fig. 2*D*). Fraction 8 produced a pattern closer to a T-banding than to an R-banding (Fig. 2*E*); T-bands were very sharp and strongly labeled; some R′-bands could still be seen, but the signal was extremely weak. Fraction 9 elicited a typical T-banding (Fig. 2*F*). As already mentioned, fraction 10 was the fraction used in previous work (11) on the localization of isochore family H3 in human chromosomes.

Hybridization with pooled fractions 7–9 showed an R-banding pattern perfectly superimposable to that obtained by standard cytogenetic techniques (Fig. 3).

Several additional observations deserve to be reported at this point. (*i*) Centromeres and constitutive heterochromatin

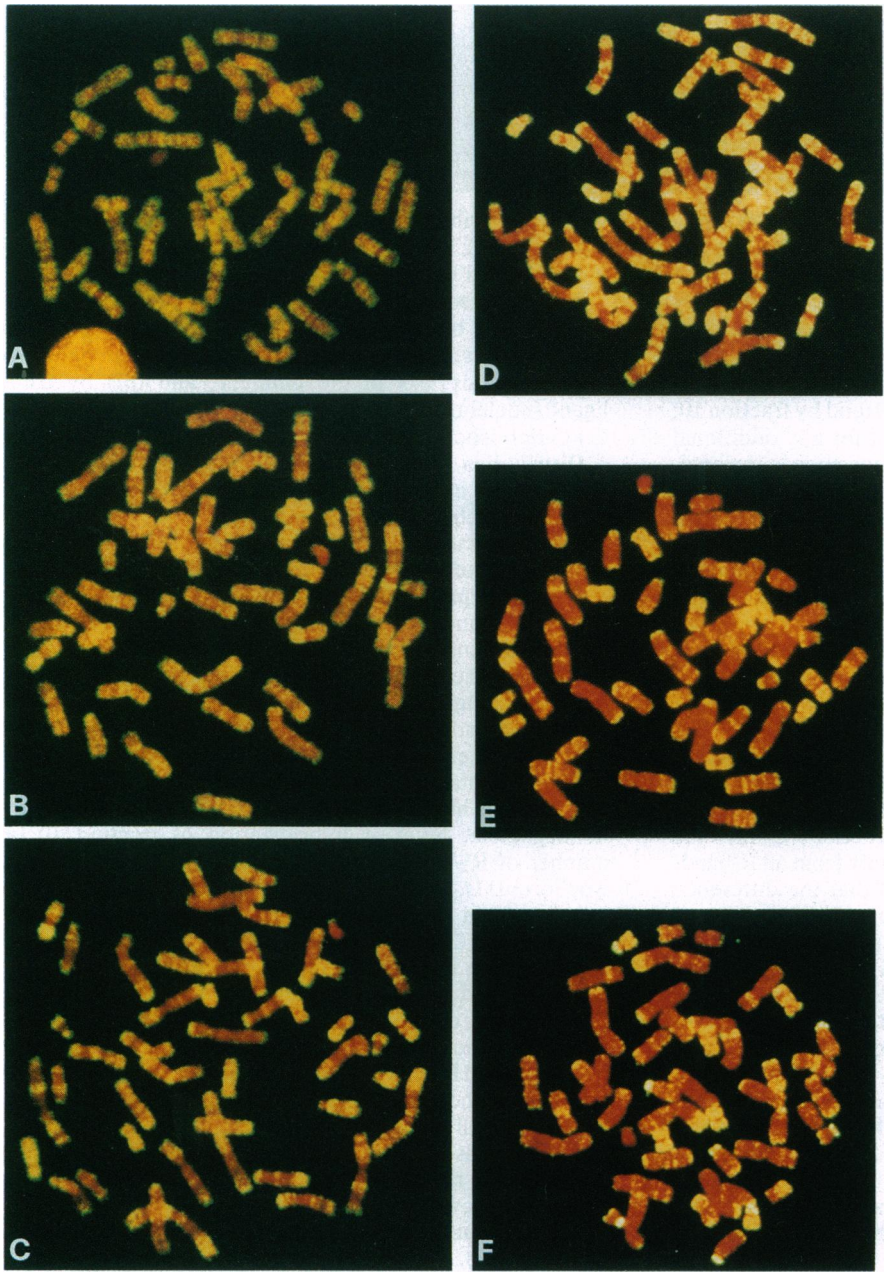

FIG. 2. *In situ* hybridization of aliquots of human DNA fractions on metaphase chromosomes. (*A*) Fractions 1–4, corresponding to the isochore families L1 + L2. (*B–D*) Fractions 5–7, corresponding to the isochore family H1. (*E* and *F*) Fractions 8 and 9, corresponding to isochore family H2. Detection of biotinylated probes was obtained with fluorescein isothiocyanate-conjugated avidin (yellow), and chromosomes were stained with propidium iodide (red).

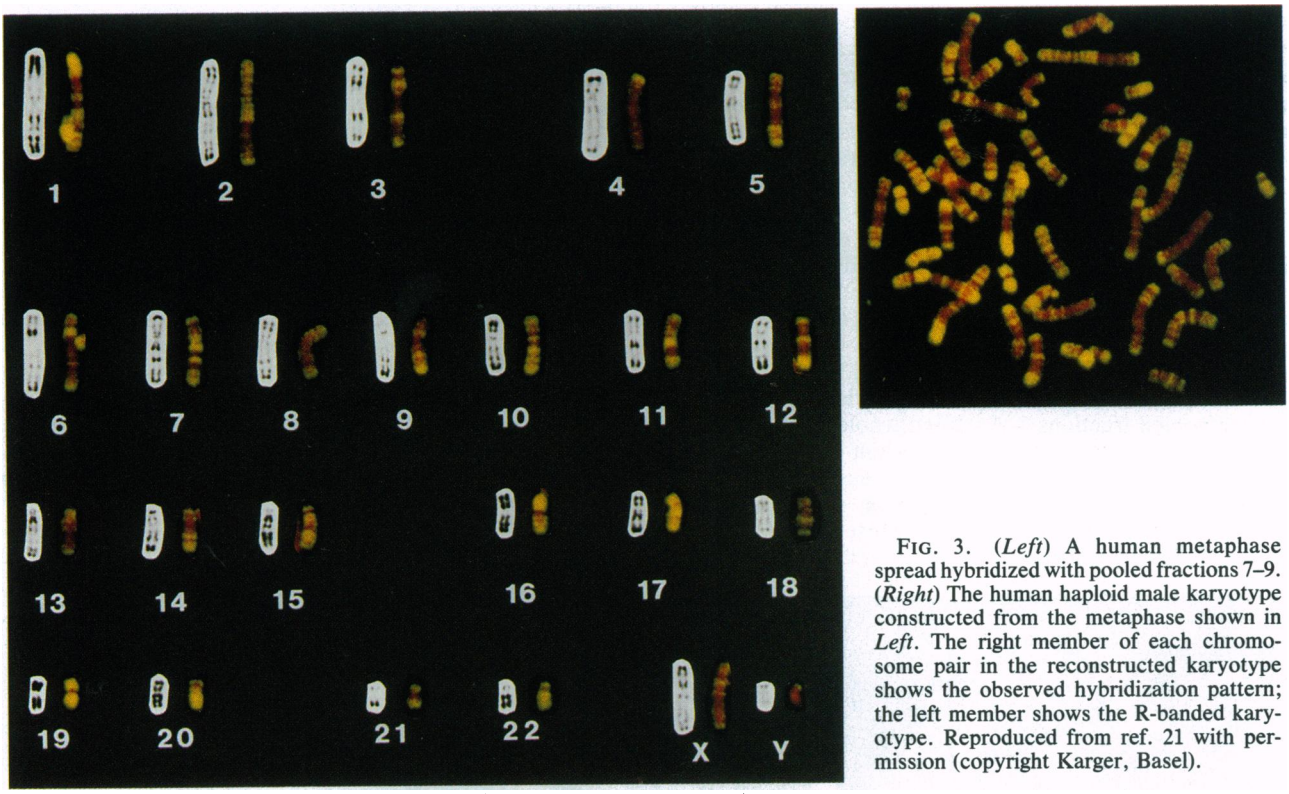

FIG. 3. (*Left*) A human metaphase spread hybridized with pooled fractions 7–9. (*Right*) The human haploid male karyotype constructed from the metaphase shown in *Left*. The right member of each chromosome pair in the reconstructed karyotype shows the observed hybridization pattern; the left member shows the R-banded karyotype. Reproduced from ref. 21 with permission (copyright Karger, Basel).

were always depleted of signals, as expected from an effective competition. (*ii*) The reproducibility of the hybridization pattern made a statistical analysis unnecessary. Since large amounts of "single-copy" sequences were hybridized, every chromosome region endowed with homology for the probes was labeled, with no artefactual lack of signals. (*iii*) Ribosomal DNA in acrocentric chromosomes was weakly labeled by fractions 5–8 but strongly by fraction 9 (and by fraction 10; see also ref. 11). (*iv*) There was no need for any additional chromosome banding to recognize chromosomes in the analysis of signal distribution because the distribution of hybridization signals practically coincided with R- or T-bands (except for the hybridization of fractions 1–4). (*v*) Concerning chromosome 21, which was already studied in its compositional map at the molecular level (10), we observed an excellent correspondence with *in situ* hybridization results, fractions 8 and 9 being prevalent in more telomeric positions of the 21q22 band compared with fractions 5, 6, and 7.

DISCUSSION

Isochores and Chromosomal Bands. The key observation made in this work is that different isochore families have a different distribution in human metaphase chromosomes. Concerning the G+C-rich fractions (fractions 5–11), the hybridization pattern changed progressively from an R-banding to a T-banding. One should note (*i*) that the difference between the results obtained by fractions corresponding to H1 and H2 isochores is very strong (compare *D* and *E* in Fig. 2), whereas the difference concerning H2 and H3 isochores is weak (compare Fig. 2*F* with figure 2 in ref. 11); (*ii*) that the present results clearly show that different chromosome bandings are correlated with the base composition of isochores and not with the different concentration (22, 23) of different repetitive sequence families in different isochore families [indeed, hybridization concerned single-copy sequences, which represent 70%, 54%, and 46% of DNA in isochore families L1 + L2, H1, and H2, respectively (24)]; and (*iii*) that previous results on the chromosomal location of short and long interspersed repetitive elements (SINES and LINES) on R-bands and G-bands, respectively (25, 26), are in agreement with the distribution of those repeated sequences in isochore families (22, 23) and with the location of isochore families in chromosomal bands (present work).

A better resolution of the results as presented here can be obtained (*i*) by using the more elongated prometaphase chromosomes; (*ii*) by using higher molecular weight DNA, which spreads less in the gradient; and (*iii*) by using a ligand/nucleotide value providing a higher resolution of G+C-rich isochore families.

Distribution of Isochore Families in Chromosomal Bands. We will now attempt to assess the relative concentration of different isochore families in G-, R′- and T-bands. In the following discussion, all percentages of isochore families are referred to the total human genome.

T-bands represent 15% of the genome on the basis of the ratio of T-bands over all bands, size differences of bands being taken into account. Essentially, the same estimate is reached on the basis of thermal denaturation (6) or chromomycin staining (7).

Since the isochores of the H3 family represent at most 5% of the genome, they can at most correspond to one-third of the DNA contained in T-bands. In fact, not all H3 isochores are present in T-bands, as indicated by the lower, yet nonnegligible, level of hybridization of fraction 10 on a number of R′-bands, whereas G-bands are depleted of H3 isochores (11). Along the same line, very recent results on the compositional mapping of the q26-qter region of the X chromosome (27) showed that a region corresponding to H3 isochores can be detected in the telomeric R-band, which is not a T-band. This result suggests that other telomeric R′-bands may contain H3 isochores, even if they do not show up cytogenetically as T-bands. The reasons for this are not understood. However, it should be stressed (*i*) that only one-third of the H3 region located in Xq28 has been explored compositionally, this situation permitting the possibility of G+C-poorer isochores being interspersed with H3 isochores; and (*ii*) that the region is flanked by sequences that are particularly low in G+C.

T-bands must comprise, therefore, also isochores from other families. This can be understood if one considers that

in the two cases in which compositional mapping data at the molecular level exist (21qter and Xqter; see refs. 10 and 27, respectively), H3 isochores are restricted to the subtelomeric region of these bands. Since isochores from the L1 and L2 families seem to be very scarce in T-bands (as shown by the very low degree of hybridization of fractions 1–4), the other isochores must essentially be those of the H1 or H2 families, or both. On the basis of the results by Saccone *et al.* (11), up to one-fourth of H3 isochores might be localized in R′-bands, leaving 3.5–4% of H3 DNA in T-bands. By assuming (on the basis of a comparison of the hybridization results as obtained with H2 and H3, respectively; see Fig. 2*F* and ref. 11) that H2 isochores are partitioned between R′- and T-bands like H3 isochores, 7.5% of H2 isochores might be localized in T-bands, the rest of the DNA present in T-bands (4%) being formed by H1 isochores.

It should be noted that the intensity of individual T-bands, as estimated both by thermal denaturation and by chromomycin staining, covers a wide range. The different intensity of T-bands may be due to different G+C levels and/or to different extensions of regions of G+C-richness on the chromosome bands. However, the first feature is certainly predominant, as indicated by the fact that R′-banding is elicited by DNA fractions that have a lower G+C level than those just producing T-bands. This suggests that different intensities of T-bands, as detected by Dutrillaux (6), Ambros and Sumner (7), and Saccone *et al.* (11), correspond to different proportions of isochores from the H1, H2, and H3 families.

R′-bands represent about 35% of all bands (this value being calculated by subtracting 15% of the genome represented by T-bands from the 50% of the genome representing all R-bands). R′-bands are essentially formed by H1 isochores and by part of isochores L1 and L2, the contribution of H2 and H3 being very small (corresponding to 4% of the DNA; see above). A quantitative assessment of the relative contribution of H1 and L1 + L2 isochores to R′-bands can be attempted as follows. Isochores of the H1 family represent about 22% of the genome, a small part of which is present in G-bands (see below) and in T-bands. If the amount of H1 isochores not present in R′-bands is assumed to be 5% (see below), the relative contribution of H1 and L1 + L2 to R′-bands would be 17% and 14%, respectively. Thus, the best present estimate is that R′-bands contain almost equal amounts of isochores from the H1 and L1 + L2 families. This accounts for the low average G+C level of third codon positions of genes located in R-bands (12, 15) and for the fact that, while R-bands replicate early and G-bands replicate late in the cell cycle (28), both G+C-rich and G+C-poor isochores replicate early and late in the cell cycle (29).

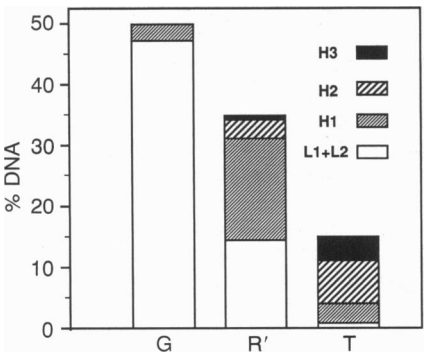

FIG. 4. A scheme of the relative amounts of isochore families L1 + L2, H1, H2, and H3 in G-, R′- and T-bands.

G-bands are formed essentially by L1 and L2 isochores, representing 48% of all bands [62% of L1 + L2 isochores less the amount (14%) present in R′-bands], which, together with about 1–2% of H1 isochores, match the 50% of chromosomal DNA represented by G-bands. Indeed, small amounts of isochores of the H1 family are also present in G-bands, as indicated by the appearance of "thin" R-bands detected in G-bands at high resolution (9).

Although the above figures can only be approximative, they provide estimates that appear not to be affected by any major inconsistency. Needless to say, these estimates can be refined by further work. A schematic presentation of the results reported here is shown in Fig. 4. A further step in this type of analysis would be an assessment of different isochore families in different chromosomal bands. As already indicated, differences are known to exist in different R′- and T-bands (see, for example, refs. 10 and 27), the scheme of Fig. 4 only concerning the average distribution.

An important implication of the present results is that they provide an indication of gene distribution over chromosomal bands. Indeed, gene concentration is correlated with the G+C level of isochores (13).

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