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Identification of the gene-richest bands in human chromosomes

Salvatore Saccone [a,b], Simone Cacciò [1,a], Jun Kusuda [2,a], Letizia Andreozzi [b], Giorgio Bernardi [a,*]

[a] *Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2 Place Jussieu, 75005 Paris, France*
[b] *Dipartimento di Biologia Animale, Università di Catania, via Androne 81, 95124 Catania, Italy*

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Abstract

The human genome is a mosaic of isochores, long DNA segments which are compositionally homogeneous and which can be partitioned into five families, L1, L2, H1, H2 and H3, characterized by increasing GC levels and by increasing gene concentrations. Previous investigations showed that in situ hybridization with a DNA fraction derived from the GC-richest and gene-richest isochores of the H3 family produced the highest concentration of signals on 25 R(everse) bands that include the 22 most thermal-denaturation-resistant T(elomeric) bands, a subset of R bands. Using an improved protocol for in situ hybridization and cloned H3 isochore DNA, we have now shown (i) that the number of bands which are characterized by strong hybridization signals, and which are here called T or $H3^+$, is 28; (ii) that 31 additional R bands, here called T′ or H3* bands, also contain H3 isochores, although at a lower concentration than $H3^+$ bands; and (iii) that the remaining R bands (about 140 out of 200, at a resolution of 400 bands), here called R″ or $H3^-$ bands, do not contain any detectable H3 isochores. $H3^+$ and H3* bands contain all the gene-richest isochores of the human genome. The existence of three distinct sets of R bands is further supported (i) by the different compositional features of genes located in them; (ii) by the very low gene density of chromosomes 13 and 18, in which all R bands are $H3^-$ bands; (iii) by the compositional map of a H3* band, Xq28; (iv) by the overwhelming presence of GC-rich and GC-poor long (>50 kb) DNA sequences in $H3^+$/H3* and in $H3^-$/G bands, respectively; and (v) by the large degree of coincidence of $H3^+$ and H3* bands with CpG island-positive bands. These observations have implications for our understanding of the causes of chromosome banding and provide a classification of chromosomal bands that is related to GC level (and to gene concentration).

Keywords: Giemsa and Reverse bandings; In situ hybridization; Isochores

1. Introduction

The human genome is a mosaic of isochores, long DNA segments, which are compositionally homogeneous and can be partitioned into five families, namely two GC-poor families (L1 and L2, collectively called L), representing about 62% of the genome, and three GC-rich families (H1, H2 and H3), representing about 22%, 9% and 3–4% of the genome, respectively, the remaining 3–4% being formed by satellite and ribosomal DNAs (Bernardi et al., 1985; see Bernardi, 1995, for a recent review). Gene concentration parallels GC levels (GC is the molar fraction of guanine + cytosine in DNA), being low in GC-poor isochores and increasingly high in increasingly GC-rich isochores (Bernardi et al., 1985; Mouchiroud et al., 1991; Bernardi, 1995; Zoubak et al., 1996). In situ hybridization of compositional DNA fractions corresponding to different isochore families provides, therefore, information not only on the correlation between isochores and chromosomal bands, but also on the gene distribution in chromosomes.

In the first investigation of this kind (Saccone et al., 1992), it was shown that the hybridization of a DNA fraction derived from H3 isochores produced the highest concentration of signals essentially on two largely coinci-

* Corresponding author. Fax +33 1 44277977; e-mail: bernardi@citi2.fr

[1] Present address: Laboratorio di Parassitologia, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Roma, Italy.

[2] Present address: National Institute of Health, 1-23-1 Toyama-cho, Shinjuku-ku 162, Tokyo, Japan.

Abbreviations: BAMD, 3,6-bis (acetato-mercurimethyl)-1,4 dioxane; bp, base pair(s); DAPI, 4,6 diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; GC, molar fraction of guanine + cytosine in DNA; GC_3, GC of third codon positions, human Genome Data Base; kb, kilobase(s); YAC(s), yeast artificial chromosome(s).

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dent subsets of R(everse) bands i.e. of G(iemsa) negative bands. These two subsets were (i) the T(elomeric) bands (Dutrillaux, 1973), which are the most heat-denaturation-resistant R bands; and (ii) the chromomycin A3-positive, DAPI-negative bands (Ambros and Sumner, 1987), which are the GC-richest bands of human chromosomes (DAPI is 4,6-diamidino-2-phenylindole). Moreover, in situ hybridization of H3 isochore DNA (Saccone et al., 1992) established that T bands comprise GC-rich, gene-rich, single-copy DNA, because the contribution of repetitive DNAs was suppressed by competition with excess unlabeled total human DNA and Alu sequences, the latter being the most abundant type of short interspersed elements (or SINEs; Singer, 1982). This ruled out the possibility, which was still open, that T bands correspond to satellite DNA. It should be noted that Saccone et al. (1992) observed a number of R bands showing medium or low concentrations of signals, in addition to those showing the highest concentration of signals.

In subsequent investigations, the chromosomal locations of the other isochore families L1+L2, H1 and H2 were studied (Saccone et al., 1993) using an improved competition protocol with excess Cot1 DNA. In contrast to the earlier experiments (Saccone et al., 1992), the new protocol did not require a statistical evaluation of the signals because all metaphases showed signals on the same bands. This established (i) that T bands contain not only H3 isochores, but also H2 and some H1 isochores; (ii) that R′ bands (namely R bands exclusive of T bands) are formed, on the average, to almost equal extents by H1 and L isochores, H2 and H3 isochores being only rarely present; and (iii) that G bands essentially consist of L isochores, H1 isochores being present at low levels.

In the present work, we have reassessed the distribution of H3 isochores on human chromosomes, by using cloned H3 isochore DNA and the improved competition conditions of Saccone et al. (1993).

2. Materials and methods

2.1. DNA fractionation and H3 library construction

High molecular weight DNA was prepared from a human placenta and fractionated in a Cs_2SO_4/BAMD density gradient as described (Cuny et al., 1981; BAMD is 3,6-bis(acetato-mercurimethyl)-1,4-dioxane). Compositional DNA fractions were submitted to analytical centrifugation as described (Macaya et al., 1976). The resulting profiles are illustrated in Fig. 1. DNA from fraction 8, that is derived from the H3 isochore family, was used for the construction of the library. Briefly, 1 μg of DNA was partially digested with Sau3AI, ligated into the lambda vector GEN-tm 11 (Promega), and used to infect the SURE strain of *E. coli* (Stratagene). The library obtained contained about 5×10^4 recombinant phages with an average insert size of 15 kb, namely an estimated six H3 isochore equivalents (taking the relative amount of H3 isochores as 3.5%, and the human genome size as 3.4×10^9 bp).

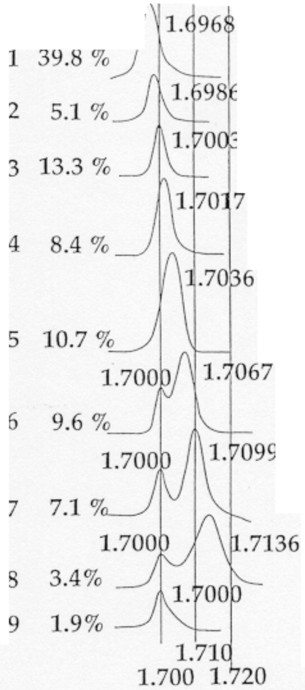

Fig. 1. Analytical CsCl profiles of human DNA fractions obtained by preparative centrifugation in a Cs_2SO_4/BAMD density gradient. The modal buoyant density (in g/cm^3) and the relative amount of DNA of each fraction are indicated.

2.2. Chromosome preparation and in situ hybridization

Chromosome preparation and in situ hybridization were performed as described (Saccone et al., 1992, 1993).

2.3. An analysis of mapped genes

The human Genome Data Base (GDB; Release 5.6) was searched for genes mapped to individual chromosomal bands at a 400-band resolution. A total of 1302 loci were found. GenBank accession numbers of coding sequences from these loci were specified in GDB for about 600 genes. The GC_3 values (GC of third colon positions) of these genes were obtained through the ACNUC retrieval system (Gouy et al., 1985).

2.4. An analysis of long (>50 kb) mapped sequences

We searched GenBank release 94 (15 April, 1996) for human DNA sequences longer than 50 kb. Out of 36 sequences fulfilling this criterion, 24 had been mapped to chromosomal bands.

3. Results and discussion

3.1. DNA fractionation results

Fig. 1 displays the CsCl profiles of human DNA fractions obtained by preparative ultracentrifugation in a Cs_2SO_4/BAMD density gradient. Fraction 8, which is derived from the H3 isochore family, is almost identical in its modal buoyant density and relative amount ($\rho=$ 1.7136 g/cm^3; 3.4% of DNA) to fraction 10 ($\rho=1.7138$ g/cm^3; 3.5% of DNA; see Fig. 1 of Saccone et al., 1993) from the fractionation previously used (Saccone et al., 1992). As was the case for the previous fractionation, fraction 8 contained a GC-poor satellite ($\rho=1.7000$ g/cm^3) and a ribosomal DNA component. Neither of these components interfered, however, with the results to be described (see Saccone et al., 1993). The DNA from fraction 8 was used to construct a library in the lambda phage, and DNA extracted from the amplified library was used for the in situ hybridization experiments.

3.2. In situ hybridization results

Hybridization of fraction 8 DNA to human metaphase chromosomes produced, at a 400-band resolution, a banding pattern (Fig. 2) which comprised four sets of bands (see Fig. 3, which also presents the results of Saccone et al., 1992, for sake of comparison): (i) 28 bands, here called T or H3$^+$, showed strong hybridization signals fully covering them, and comprised the T bands and the chromomycin A3-DAPI bands previously described (Dutrillaux, 1973; Ambros and Sumner, 1987). (ii) 31 bands, called here T′ or H3*, showed medium or weak hybridization signals which covered them only partially and corresponded to a subset of R′ bands (i.e., R bands exclusive of T bands; see Saccone et al., 1993). (iii) Ribosomal DNA in acrocentric chromosomes was strongly labelled (Fig. 2) because of the presence of ribosomal DNA in fraction 8 (see above). (iv) The other 140 or so R′ bands, here called H3$^-$ bands, and G bands showed a very low level of hybridization or no hybridization. On the basis of isochore hybridization, G bands may also be designated as L bands. Table 1 summarizes the proposed classification of chromosomal bands (see also Discussion). Table 2 lists the H3$^+$ and H3* bands, as observed in the present work at a resolution of 400 bands. They are compared with previous results (Dutrillaux, 1973; Ambros and Sumner, 1987), as deduced from Table 1 of Saccone et al. (1992).

As far as H3$^+$ bands are concerned, their number, 28, as determined in the present work, is slightly higher than that, 25, previously reported for bands strongly positive for H3 DNA (Saccone et al., 1992), and than that, 26, reported for chromomycin A3-positive DAPI-negative bands (Ambros and Sumner, 1987), whereas it is definitely higher than that, 22, of the T bands, as originally described (Dutrillaux, 1973). This stresses the fact that T and H3$^+$ bands are not really synonymous, even if, for the sake of simplicity and current usage, they will be used interchangeably in this paper.

The existence of R bands showing medium and weak intensities of hybridization signals with H3 DNA, now called T′ or H3* bands, was first reported four years ago (Saccone et al., 1992). At that time, these bands, which coincided (see Table 1 of Saccone et al., 1992) with some weak bands obtained by T banding (Dutrillaux, 1973) or by chromomycin A3-DAPI staining (Ambros and Sumner, 1987), were tentatively attributed to hybridization of DNA from H2 isochores that was present in the H3 DNA fraction used. This interpretation can now be rejected on the basis of two independent reasons. First, the H3 DNA fractions show very little DNA corresponding to the buoyant density of H2 isochore DNA. Second, a comparison of the data of Fig. 2 from Saccone et al. (1993) with the present ones (Fig. 3) indicate that H2 DNA has a chromosomal distribution very similar to that of H3 DNA. Indeed, only very few bands, 3q29, 6q27, 13q34 and 20p13, appear to be H2 positive and H3 negative. Moreover, the compositional map of Xq28 has provided a specific example of the clustering of H2 and H3 isochores in a T′ or H3* band (De Sario et al., 1996). One should, therefore, conclude that the results of Saccone et al. (1992; see Fig. 3), concerned H3 DNA hybridization as the present ones do. The present results indicate, however, that some H3* bands were either missed or underestimated in hybridization intensity by Saccone et al. (1992; see Fig. 3). This was due to the strong suppression of H3 signals due to using total human DNA as a competitor, an unfavorable experimental condition which was subsequently eliminated by using Cot1 DNA (Saccone et al., 1993; and present work).

3.3. Distribution of genes mapped to chromosomal bands

The distribution of 1302 genes localized in chromosomal bands showed that they were mainly located in H3$^+$ and H3* bands, as expected from the correlation between gene concentration and GC level in isochores (Mouchiroud et al., 1991; Bernardi, 1995; Zoubak et al., 1996). These results are not shown because they lead to the assignment of 22.5% genes to G bands and 77.5% to R bands, in essential agreement with a previous estimate based on 1000 genes (Craig and Bickmore, 1993). The current data on the distribution of genes

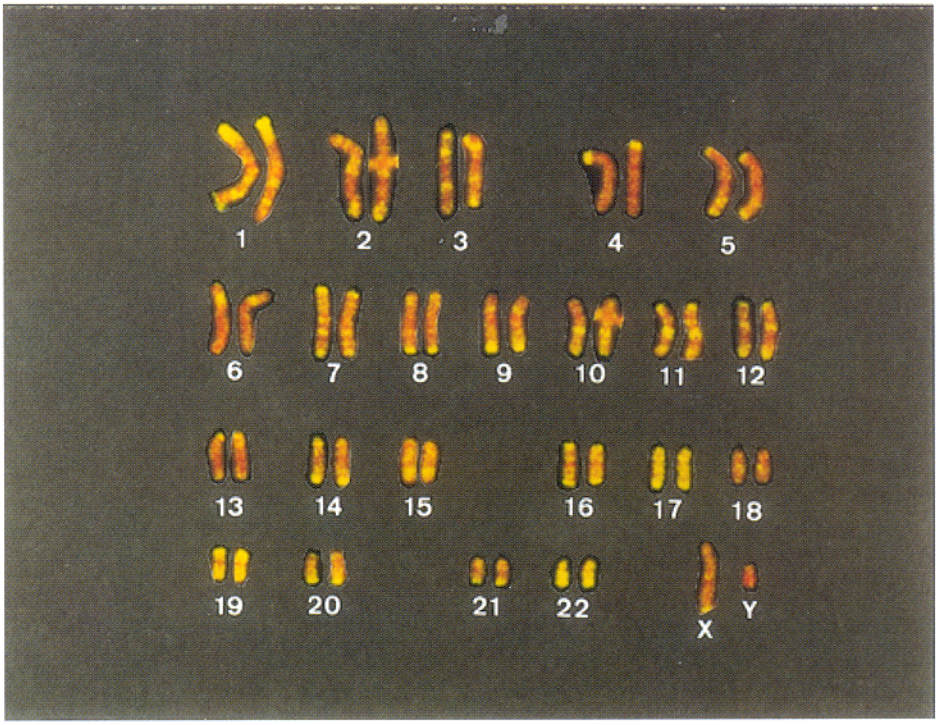

Fig. 2. Human haploid male karyotype constructed from a single metaphase hybridized with fraction 8 DNA (see Fig. 1). The biotinylated probe was detected with Avidin-FITC (fluorescein isothiocyanate; yellow staining) and chromosomes were stained with propidium iodide (red staining).

mapped to chromosomal bands indicate, in addition, that gene densities decrease from H3$^+$ to H3*, then to H3$^-$ and, finally, to G bands (not shown).

It was pointed out before (Bernardi, 1993) that chromosomes 19 and 22, which are richest in H3$^+$ bands (see Fig. 3), also are the chromosomes that are richest in genes (McKusick, 1991; the apparent high gene density of the X chromosome is the consequence of the accumulation of studies on this chromosome). The other end of the compositional spectrum of R bands can now be observed in H3$^-$ bands. Thus, chromosomes 13 and 18, the only ones containing neither H3$^+$ nor H3* bands but only H3$^-$ bands, and the only ones (except for the smallest autosome, chromosome 21) showing trisomy compatible with live birth, are the two gene-poorest chromosomes (McKusick, 1991). Interestingly, more recent genome data base releases and chromosomal assignments of partial cDNA sequences (Polymeropoulos et al., 1993; Fukushima et al., 1994; Murakawa et al., 1994) provide further support for these conclusions.

3.4. Compositional properties of mapped genes

Fig. 4 shows the distribution of GC_3 values from about 600 coding sequences which were localized on G, H3$^+$, H3* and H3$^-$ bands. The average GC_3 values were: (i) 60% for 136 genes localized in G bands; (ii) 59% for 150 genes localized in H3$^-$ bands; (iii) 65% for 154 genes localized in H3* bands; and (iv) 70% for 169 genes localized in H3$^+$ bands. In all cases, standard deviations were 15–16%. GC_3 values of genes from H3$^+$, H3* and H3$^-$ bands are different, the GC_3 values of genes from H3$^-$ and G bands being close to each other. Because of the correlation between GC_3 levels and GC levels of the isochores embedding the corresponding gene (Bernardi et al., 1985; Aïssani et al., 1991; Clay et al., 1996), one should conclude that the different subsets of R bands have different compositions.

While previous work (Ikemura and Wada, 1991; De Sario et al., 1991), performed on a smaller data set (about 200 instead of 600 genes), showed GC_3 differences among genes located in G, R and T bands, respectively, Fig. 4 shows, in addition, that genes mapped to H3$^+$ bands and to H3* bands have a different, and higher, average GC_3 value compared to genes mapped to H3$^-$ and G bands. Moreover, genes located in H3* bands are compositionally intermediate between those located in H3$^+$ bands and in H3$^-$ bands, as expected from the different proportion of different isochores in those bands. These results provide independent support for the existence of three subsets of R bands, H3$^+$, H3* and H3$^-$.

The wide distributions of GC_3 values within each class of bands could be due to misassignments of genes

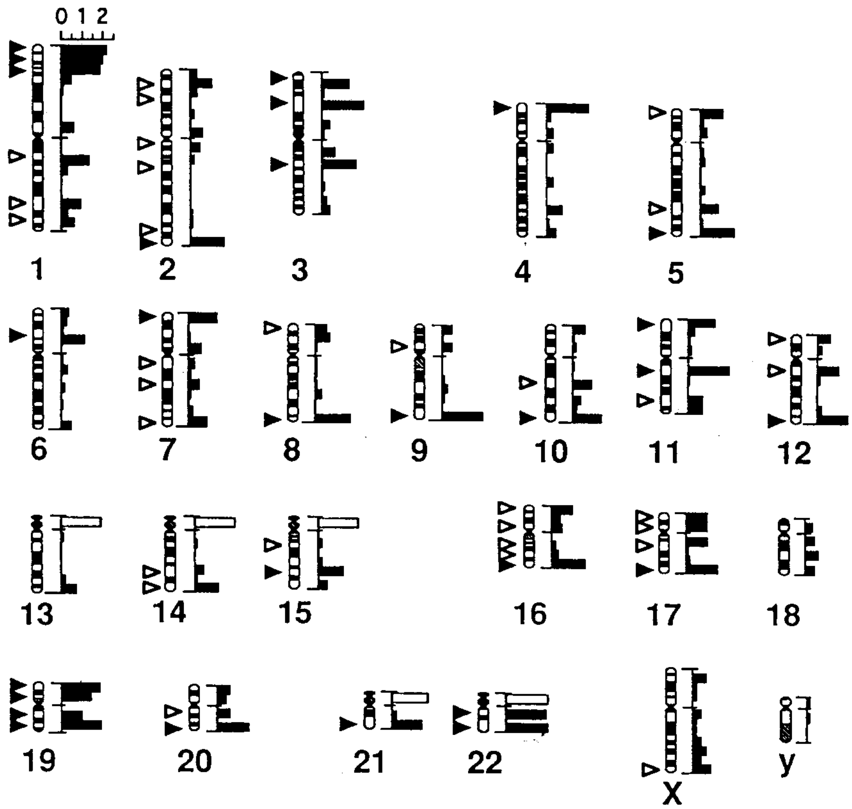

Fig. 3. G banded human male karyotype (at a resolution of 400 bands) showing the bands detected in the present work by hybridization of a H3 isochore probe (arrows on the left of each chromosome; solid arrows indicate the strong signals of $H3^+$ bands, open arrows the medium or weak signals of H3* bands), as compared with the histogram (Saccone et al., 1992) of hybridization signals with H3 isochores (solid bars on the right of each chromosome; empty bars correspond to ribosomal DNA; the histogram scale is the percentage of the total number of hybridization signals).

Table 1
Proposed classification of chromosomal bands

Bands	H3 isochore hybridization	Isochores present[a]	Gene concentration
R bands			
T-bands ($H3^+$)	strong	**H3+H2**+*H*1+*L*	++++
R′-bands			
T′-bands (H3*)	medium–weak	H3+H2+H1+L	+++
R″-bands ($H3^-$)	undetectable	*H*1+L	++
G bands (L)	undetectable	L+*H*1	+

[a]Bold-type indicates the predominant isochore families, italics the minority isochore families. H3* bands were called H3 bands by Bernardi (1995).

to chromosomal bands and/or due to compositional heterogeneity within bands. In order to clarify this point, two subsets of genes were analyzed: (i) 38 genes located in G bands of chromosomes 19 and 22 (which contain an unusually high proportion of $H3^+$ bands) showed a higher average GC_3 value, 67%, than those located in the other G bands, 57.5%; since G bands are remarkably homogeneous in composition (Gardiner et al., 1990; Saccone et al., 1993; Pilia et al., 1993; Bernardi, 1995), this suggests misassignments of genes from flanking $H3^+$ bands to G bands, at least in some chromosomes and in some chromosomal regions. (ii) 46 genes localized on 4q, a chromosomal arm which only comprises G and $H3^-$ bands, showed an average GC_3 of only $44 \pm 9\%$; since this value is noticeably lower, and shows a lower standard deviation, than the average GC_3 ($60 \pm 15\%$) of the genes contained in the other G and $H3^-$ bands (several of which are contiguous to $H3^+$ and H3* bands), this suggests again that errors in assigning genes to specific bands may account for the high standard deviation of GC_3 values. In contrast, compositional heterogeneity within bands should only play a minor role in spreading GC_3 values, at least in G and $H3^-$ bands, since these bands have a low compositional heterogeneity (see above).

The low GC levels found in $H3^-$ bands, which correspond to the majority of R bands, are in agreement with the fact that differences in base composition of DNA from G and R bands are small (Holmquist et al., 1982; Stephens et al., 1990).

Table 2
R bands hybridizing H3 isochores[a]

	H3$^-$ bands	H3$^-$ bands
chr 1	**p36.1**$^-$, **p36.3**$^-$, p34$^-$	q21, q32, q42^{-b}
chr 2	q37$^-$	p23, p21$^-$, q11.2, q21, q35
chr 3	p25, **p21**$^-$, q21	–
chr 4	**p16**$^-$	
chr 5	q35	p15.3$^-$, q31$^-$
chr 6	p21.3$^-$	
chr 7	**p22**$^-$	q11.2$^-$, **q22**, **q36**
chr 8	**q24.3**	p23$^-$
chr 9	**q34**$^-$	p13
chr 10	**q26**$^-$	q22^{-c}
chr 11	**p15**, **q13**$^-$	q23
chr 12	q24.3	p13, q13
chr 13	–	–
chr 14	–	q24, **q32**$^{-c}$
chr 15	q24$^-$	q15^{-b}
chr 16	**q24**$^-$	**p13.3**$^{-c}$, p11.2, q13$^-$, **q22**
chr 17	**q25**$^-$	p13$^-$, p11.2, q21$^-$
chr 18	–	–
chr 19	**p13.3**$^-$, **p13.1**$^-$, q13.1$^-$, **q13.3**$^-$	
chr 20	**q13.3**$^-$	q11.2
chr 21	**q22**$^-$	–
chr 22	**q11.2**$^-$, **q13**$^-$	–
chr X	–	q28$^-$
chr Y	–	–

[a]T bands of Dutrillaux (1973) are in bold type; chromomycin A3-DAPI bands of Ambros and Sumner (1987) are underlined [only bands classified as +++ or ++ in table 1 of Saccone et al. (1992) are taken into account]. Minus signs indicate bands not covered, or poorly covered, in the YAC contig maps of Gemmill et al. (1995) for chromosome 3, of Krauter et al. (1995) for chromosome 12, of Collins et al. (1995) for chromosome 22 and of Chumakov et al. (1995) for other chromosomes.
[b]These H3* bands are very weak.
[c]These H3* bands are stronger than the average.

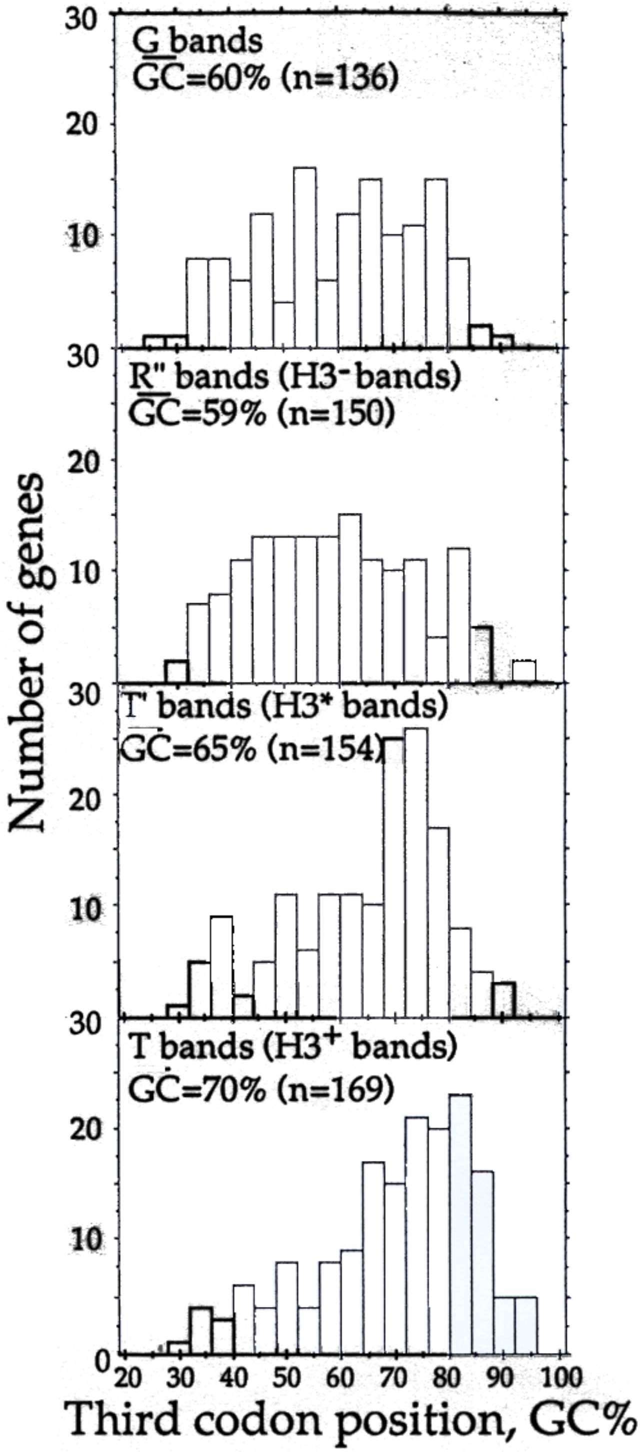

Fig. 4. Histograms of GC_3 (the GC levels of third codon positions) of human genes localized in G, H3$^-$, H3$^+$ and H3* bands. The average GC content and the relative number of genes for each band type is indicated.

3.5. *Compositional properties of mapped long sequences*

All very long (>50 kb) sequences above 50% GC were mapped to H3$^+$ or H3* bands, whereas those below 50% GC were mapped to G or H3$^-$ bands with only two sequences mapping to H3$^+$ bands. Fig. 5 shows a histogram concerning these results.

3.6. *A comparison of H3$^+$ and H3* bands with CpG island-positive bands*

CpG island concentration increases in compositional DNA fractions of increasing GC level and parallels gene concentration (Aïssani and Bernardi, 1991a, 1991b). A correlation should, therefore, be expected between in situ hybridization signals as obtained with compositional DNA fractions of increasing GC level (Saccone et al., 1992, 1993; and present work) and with CpG islands. Indeed, HpaII sites, a large number of which are in CpG islands, are clustered in R bands (Sentis et al., 1993) and especially in T bands (Ferraro et al., 1993). The latter also expectedly show the highest concentration of CpG

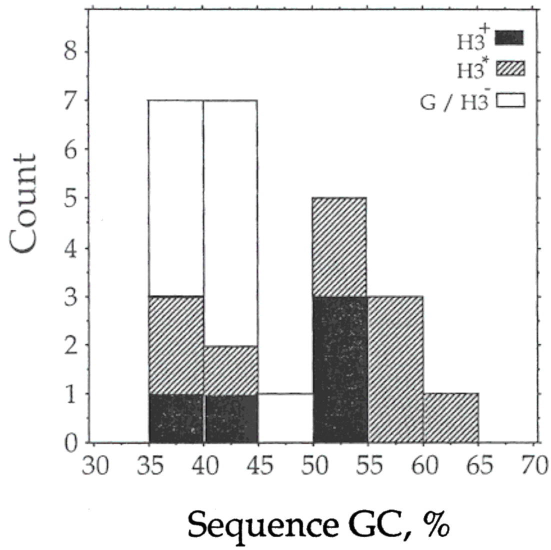

Fig. 5. Histogram of long DNA sequences (> 50 kb) characterized by different GC levels mapped on H^+, H3* and $H3^-$/4 chromosomal bands.

islands (Craig and Bickmore, 1994). A detailed comparison between the latter and the present data showed that while $H3^-$ bands are CpG island-negative, CpG island hybridization revealed not only $H3^+$ bands, but also most H3* bands, with only a few exceptions: five H3* bands present on chromosome 2, and one, Xq28, present on the X chromosome, were missed, out of a total of about 60 bands. In the latter case, this happened in spite of the fact that H3* band Xq28 is characterized by a high CpG island density in its H2 and H3 isochores (see De Sario et al., 1996). Conversely, two bands not detected by H3 isochore hybridization were detected by CpG island hybridization. One of them, on 6q, was an H2 isochore band, the other one was located on 9q.

4. General implications

4.1. Base composition and chromosomal bands

The results presented above show that the classification of R bands into three families, $H3^+$, H3*and $H3^-$, rests on a compositional basis (at the same time, being a classification related to gene concentration). In spite of such compositional differences, all three families of bands are Giemsa-negative, R-bands. In contrast, $H3^-$ bands and G bands, in spite of being closer compositionally than $H3^-$, H3* and $H3^+$ bands are, belong into two cytogenetically very distinct classes of bands, R and G. This suggests that the basic difference between G and R bands is not simply due to differences in base composition, contrary to a widespread explanation (see Comings, 1978). An alternative interpretation of the differences between G and R bands is that G bands are compositionally more homogeneous, endowed with a more closed chromatin structure and with a higher DNA packing than $H3^-$ bands (Bernardi, 1989). This explanation is compatible with a recent model in which the basis of banding patterns is the differential folding of the AT-rich scaffold and packing of DNA loops in G and R bands (Saitoh and Laemmli, 1994).

4.2. The classification of chromosomal bands

The classical viewpoint, which has prevailed for the past twenty years, of two sets of chromosomal bands, G and R, was modified by the finding of the highest GC (and gene) levels in T bands (Bernardi, 1989; Gardiner et al., 1990; Saccone et al., 1992). Because of their specific properties, these bands were, therefore, to be distinguished from the rest of R bands (called R′ bands by Saccone et al., 1993). The present work indicates that not two but three sets of R bands, $H3^+$, H3* and $H3^-$ bands, can be visualized on the basis of the relative amount of given isochore families (and of the corresponding gene densities), as indicated in Table 1.

Two series of remarks about band classification are appropriate at this point. The first one concerns the T bands of Dutrillaux (1973). These elusive bands, were called Telomeric bands because the majority of the 22 bands originally described were located in telomeric positions. Our data show that 28 bands are characterized by essentially the same strong hybridization intensity with H3 isochores. This means that, if T bands are considered as synonymous with $H3^+$ bands, T bands located in telomeric positions only represent about half of all T bands. Under these circumstances, the term 'Telomeric Bands' becomes a misnomer. If this term to be kept, because of its current widespread use, it seems more appropriate to understand it as the acronym of T(hermally)-resistant bands.

The second series of comments concerns the classification of chromosomal bands in five 'metaphase chromatin flavors', corresponding to (GC-poor) G bands, GC-poor R bands (Alu-poor or Alu-rich), and GC-rich R bands or T bands (again Alu-poor or Alu-rich), which was proposed by Holmquist (1992) essentially on the basis of published results (Dutrillaux, 1973; Korenberg and Engels, 1978; Manuelidis and Ward, 1984; Ambros and Sumner, 1987; Korenberg and Rikowski, 1988; Bernardi, 1989; Gardiner et al., 1990). The 'flavor' classification has the major weakness that it goes beyond the distinction between R bands and their subset of T bands only in one respect, namely in also taking into account the Alu concentration. This is, however, a parameter of limited significance, as indicated by the much higher Alu level in the human compared to other mammalian (and even primate) genomes, as well as by the fact that mammalian and avian genomes exhibit common banding features independently of the amount and nature of interspersed repeated sequences. A detailed comparison of the classification proposed here with the 'flavor' classification (see Table 3) stresses a number of inconsistencies in the latter: (i) our 28 $H3^+$ bands comprise 18 $vAlu^+$ vGC^+, 9 Alu^- vGC^+ bands (showing that these bands may be Alu-rich or Alu-poor) and, surprisingly, even 1 Alu^- vGC^- band; (ii) our $H3^-$ bands essentially correspond to 'mundane' (namely Alu-poor, GC-poor) R bands with, however, a few Alu-rich, GC-poor bands; and (iii) our H3* bands are found in all four R band 'flavors'. It is, therefore, suggested that the 'flavor' classification be abandoned.

4.3. Chromosome maps and gene coverage

The most recent YAC contig map is said to cover about 75% of the human genome (Chumakov et al., 1995). It is difficult to judge how reliable this value is because "the actual proportion of the physical length of the genome covered is not entirely straightforward", the proportion of the genetic length covered (66%) possibly overestimating or underestimating the coverage. The question of interest here concerns the chromosomal location of the regions characterized by a missing or poor coverage on the YAC contig map. As pointed out by the authors themselves, chromosome arm 1p, and chromosomes 17 and 19 were poorly covered. A more detailed analysis of the results of Chumakov et al. (1995) shows, however, that, in fact, most $H3^+$ and H3* bands were not covered at all or poorly covered (see Table 2). Since $H3^+$ bands represent about 15% of chromosome length (Saccone et al., 1993), and H3* bands about the same percentage, these bands essentially correspond to the 25% of the genome that are not covered or poorly covered. In other words, the gene-richest regions of the human genome are not covered by the YAC contig map of Chumakov et al. (1995). The instability of YACS derived from these regions (De Sario et al., 1996) explains this situation and the fact that essentially no improvement could be obtained between the first generation physical map of the human genome (Cohen et al., 1993) and the most recent one (Chumakov et al., 1995). Under these circumstances, gene coverage is less than 50%, as estimated by Zoubak et al. (1996).

Table 3
A comparison of the classification of R bands according to metaphase chromatin flavors (Holmquist, 1992) and to isochore H3 DNA distribution (present work)[a]

		T bands (42)		R bands (108)[b]	
Holmquist (1992)		**(28)**	**(14)**	**(7)**	
Present work					
$H3^+$ bands	**(28)**	18	9	0	1
H3* bands	**(30)**[c]	10	5	3	12
$H3^-$ bands	**(140)**[b]	0	0	4	88

[a]Bold values in parentheses are the numbers of bands belonging in each class.
[b]The classification of Holmquist (1992) is based on a 300-band resolution disregarding sex chromosomes, the present one on a 400-band resolution; this difference mainly affects $H3^-$ bands.
[c]Xq28, a H3* band, was disregarded here because the sex chromosomes were not taken into account by Holmquist (1992).

5. Conclusions

The main conclusions of the present work are the following: In situ hybridization of DNA from H3 isochores, GC_3 values of genes localized in different chromosome bands, GC values of long mapped sequences and a comparison of H3-positive and CpG island-positive bands show that high gene concentrations are restricted to a relatively small number, 59, of R bands, which were identified here at the 400-band level (see Table 2), and which are very unequally distributed over human chromosomes.

The large compositional differences between the three sets of R bands, $H3^+$, H3* and $H3^-$, and the small compositional differences between the 200 G bands and the 140 $H3^-$ bands, stress the fact that G and R bands are not simply due to differences in GC levels. The band classification defined by isochores, namely $H3^+$, H3*, $H3^-$ and L bands, is directly related to two crucial and correlated parameters, namely GC levels and gene concentrations.

An analysis of the most recent YAC contig map of the human genome has shown that the great majority of the gene-richest bands are not covered by it. If this problem is taken into account, along with the large extent of chymerism and instability of YACs for the regions which are covered, the purpose of physical maps "to localize the complete inventory of human genes" (Chumakov et al., 1995) is still far from attained, contrary to widespread belief. Fortunately, however, the improvements of the genetic map using markers from H2 and H3 isochores (Gyapay et al., 1994), long-range sequencing efforts, especially of $H3^+$ and H3* isochores (such as those of Chen et al., 1996), and the use of bacterial clones (Venter et al., 1996) should lead to this goal in a relatively near future.

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