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Gene density in the Giemsa bands of human chromosomes

Concetta Federico[1], Letizia Andreozzi[1], Salvatore Saccone[1,2] & Giorgio Bernardi[3]*
[1]*Dipartimento di Biologia Animale, University of Catania, via Androne 81, 95124 Catania, Italy;* [2]*Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, via F.lli Rosselli 107, 42100 Reggio Emilia, Italy;* [3]*Laboratorio di Evoluzione Molecolare, Stazione Zoologica, Villa Comunale, 80121 Napoli, Italy; Tel: (+39) 081 5833300; Fax: (+39) 081 2455807; E-mail: bernardi@alpha.szn.it*
* *Correspondence*

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Abstract

The human genome is formed by isochores belonging to five families, L1, L2, H1, H2 and H3, that are characterized by increasing GC levels and gene concentrations. *In-situ* hybridization of DNA from different isochore families provides, therefore, information not only on the correlation between isochores and chromosomal bands, but also on the distribution of genes in chromosomes. Three subsets of R(everse) bands were identified: $H3^+$, H3* and $H3^-$, that contain large, moderate, and no detectable amounts, respectively, of the gene-richest H2 and H3 isochores, and replicate very early and early, respectively, in S phase of the cell cycle. Here, we investigated the GC levels, replication timings and DNA compaction of G(iemsa) bands. We showed that G bands comprise two different subsets of bands, one of which is predominantly composed of L1 isochores, replicates at the end of the S phase, has a higher DNA compaction relative to $H3^+$ bands and corresponds to the darkest G bands of Francke (1994). In contrast, the other subset is composed of L2 and H1 isochores, has less-extreme properties in replication and composition and corresponds to the less-dark G bands of Francke.

Introduction

Human chromosomes are characterized by different staining properties of their regions, namely by chromosomal bands. The most widely used Giemsa staining, when performed after different chromosome treatments (trypsin digestion or heat denaturation), produces specific banding patterns, the G(iemsa) and the R(everse) bands. G and R bands can also be obtained by fluorescent staining (quinacrine, DAPI), differential incorporation of 5-bromodeoxyuridine (Dutrillaux *et al.* 1976, Vogel *et al.* 1989, Drouin *et al.* 1990, Fetni *et al.* 1996) and other approaches.

In-situ hybridization of DNA derived from different isochore families (for reviews see Bernardi 1995, 2000), namely chromosomal compositional mapping, represents a different approach to chromosomal banding (Saccone *et al.* 1992, 1993). This provides, moreover, information not only on the average base composition of chromosomal bands, but also on the distribution of genes in chromosomes (Saccone *et al.* 1992, 1996, 1999), gene density being correlated with the GC level

of the chromosomal bands (Bernardi *et al.* 1985, Mouchiroud *et al.* 1991, Zoubak *et al.* 1996). In fact, the $H3^+$ bands (Saccone *et al.* 1996), containing the highest concentrations of the gene-richest H3 isochores, were so identified and shown to largely coincide with the previously identified T bands (Dutrillaux 1973). Expectedly, $H3^+$ bands are endowed with the highest CpG island concentration (Craig & Bickmore 1994, Saccone *et al.* 1996).

The R and the G bands of standard ideograms are an approximation of the real situation. Indeed, some bands, such as the $H3^+$ bands, are clearly different from other R bands (see above). Moreover, $H3^+$ bands are also characterized not only by the highest concentration of genes, and by a very high level of transcriptional activity, but also by the highest recombination frequencies and by an 'open' chromatin structure (see Bernardi 2000). On the other hand, G bands, routinely shown as dark bands in ideograms, belong, in fact, to four classes characterized by different degrees of darkness (Francke 1994).

R bands and G bands also show no overlap in replication times since they replicate in the early and late S phase, respectively (Dutrillaux *et al.* 1976, Biemont *et al.* 1978, for review see Drouin *et al.* 1994). Moreover, at low resolution, almost all chromosomal bands (277 bands) were classified into 18 replication groups (Dutrillaux *et al.* 1976). Interestingly, the $H3^+$ bands showed the earliest replicating timings (Federico *et al.* 1998).

In the present work, we localized the DNAs of the L1 and L2 isochores at a higher definition compared with previous work (Saccone *et al.* 1993) and characterized the GC levels and replication timings of G bands.

Materials and methods

DNA preparation

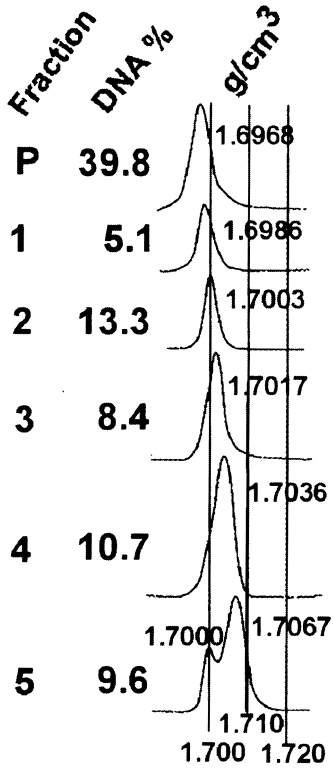
High-molecular-weight DNA was prepared from a human placenta and fractionated in a Cs_2SO_4/BAMD density gradient, at a ligand/nucleotide molar ratio of 0.14, according to Cuny *et al.* (1981); BAMD denotes 3,6-bis(acetato-mercurimethyl)-1,4-dioxane. The compositional DNA fractions used in the present work comprised the pellet and fractions 1–5 (Figure 1) from the DNA fractionation of Saccone *et al.* (1996). DNA from H3 isochores cloned in a lambda vector (Saccone *et al.* 1996) was also used.

Chromosome preparation, in-situ hybridization and GTG banding

Human metaphase chromosomes were prepared from phytohaemagglutinin-stimulated peripheral blood lymphocytes by standard cytogenetic procedures. *In-situ* suppression hybridizations were performed with 200 ng of each biotin-labelled fraction. In the case of the simultaneous hybridization of the L1 and H3 isochores, 200 ng of digoxigenin- and biotin-labelled DNA, as present in the pellet and in cloned H3 isochores, respectively, were used. In all cases, a 50-fold excess of human Cot1 DNA was added to the hybridization mixture. Hybridization conditions and detection of the biotin-labelled probes were according to Saccone *et al.* (1996). Detection of the biotin- and digoxigenin-labelled probes was obtained by avidin–TRITC and anti-digoxigenin–FITC antibody, respectively. After hybridization, chromosomes were stained with propidium iodide (single biotin-labelled probe), or DAPI (in the simultaneous hybridization of biotin- and digoxigenin-labelled probes). The GTG bands (**G** bands by **T**rypsin using **G**iemsa; see Drouin *et al.* 1994) were obtained by conventional trypsin digestion of the chromosomes before Giemsa colouration.

Nomenclature

In this paper R_{400}, G_{400}, R_{850} and G_{850} indicate R and G bands at a level of resolution of 400 and 850 bands per haploid karyotype, respectively. $H3^+_{400}$, $H3^*_{400}$ and $H3^-_{400}$ indicate R_{400} bands characterized by high, medium and undetectable levels of H3 hybridization, respectively (Saccone *et al.* 1996; see also Introduction). $H3^+_{850}$ and $H3^-_{850}$ bands are characterized by the presence or absence of the H3 isochore family. We will indicate here, as G1, G2, G3 and G4, the G_{850} bands that show the four levels of grey ranging from pale to black, as published by Francke (1994).



Isochore family %			LOCALIZATION		Figure[1]
L1	L2	H1	G bands	R bands	
30.0	9.8		G4/G3	No signals	2A and 3
	5.1		Large majority	No signals	2B
	13.3		All	H3- Weak on H3* Weak on H3+	2C
	4.8	3.6	All	H3- and H3* Weak on H3+	2D
		10.7	Some	Almost all	2E[2]
		9.6	Weak signals	Almost all	2F[3]

Figure 1. DNA fractions: compositional features and chromosomal distribution. CsCl profiles of the human DNA fractions containing the L1, L2 and H1 isochore families (modified from Saccone *et al.* 1996): fraction numbers (P stands for pellet), relative DNA content, modal buoyant density, relative amount of isochore families, and chromosomal distribution are indicated. [(1)]From Figure 2. [(2)]The same pattern was found using a probe containing H1 and a small amount of L2 isochores (Saccone *et al.* 1993). [(3)]The same pattern was obtained with H1 isochores (Saccone *et al.* 1993).

Results

Analysis of the DNA fraction

Figure 1 displays the CsCl profiles, the relative DNA content and the proportion of the isochore families in the DNA fractions (Saccone *et al.* 1996) used as probes for *in-situ* hybridizations. L1 isochores are only present in the pellet DNA; L2 isochores are distributed in the pellet and in fractions 1–3; fractions 4 and 5 contain almost exclusively DNA from H1 isochores. In the case of fraction 5 (as well as in the following fractions; see Figure 1 in Saccone *et al.* 1996), a light satellite peak corresponding to DNA from centromeric heterochromatin is present.

Identification of the GC-poorest chromosomal regions

The pellet DNA, essentially formed by L1 isochores, hybridized on a subset of G bands (Figures 2A & 3) which will be called here L1 bands and correspond to the G3 and G4 bands of Francke (1994). In contrast, the pellet DNA is almost absent in the large majority of the $H3^{+}_{400}$ and $H3^{*}_{400}$ bands. This is especially evident in chromosomes 15, 17, 19 and 22 (see Figure 3).

Interestingly, the pellet DNA represents about 40% of the total genome, most of which belongs to the L1 isochores, whereas the bands on which it is localized correspond to about 26% of all bands (as assessed from the ideogram of Francke 1994).

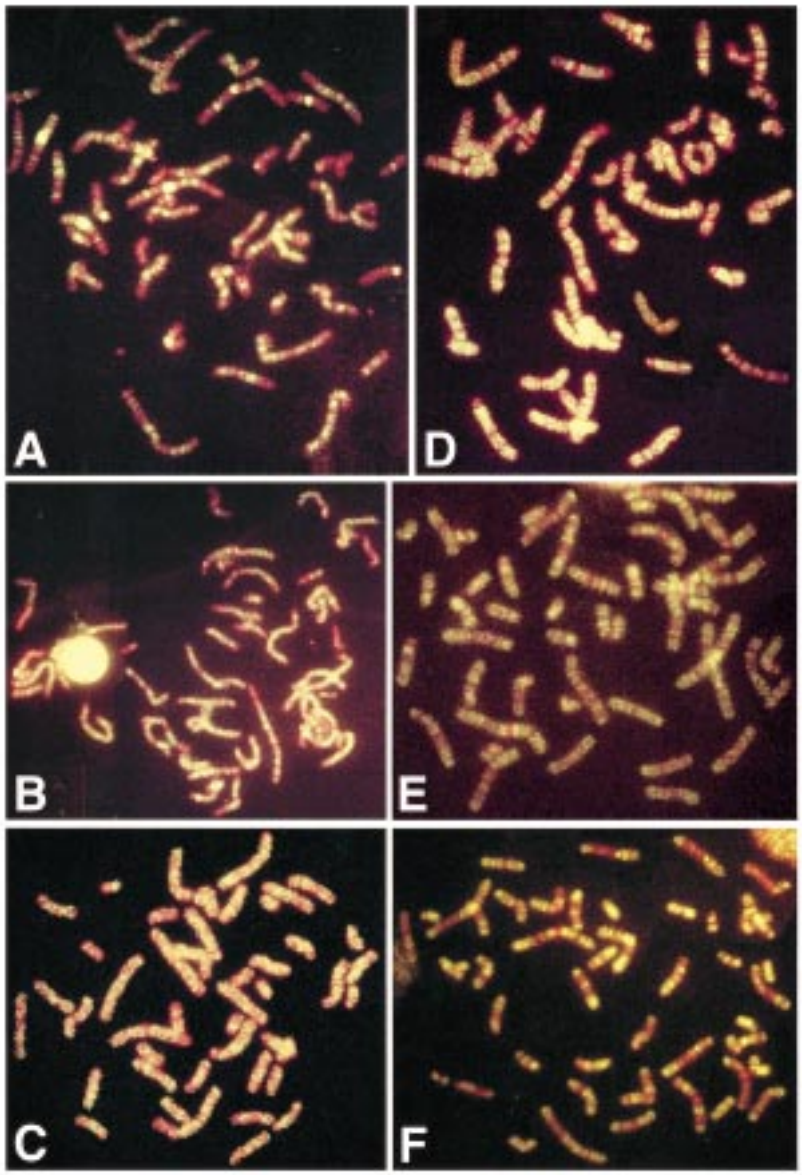


Figure 2. Localization of the L1, L2, and H1 isochore families. Human metaphase chromosomes hybridized with the DNA fractions of Figure 1. Panels show hybridizations obtained with pellet (**A**), fraction 1 (**B**), 2 (**C**), 3 (**D**), 4 (**E**) and 5 (**F**). Hybridizations were detected by avidin–FITC (green/yellow signals). Chromosomes were stained with propidium iodide.

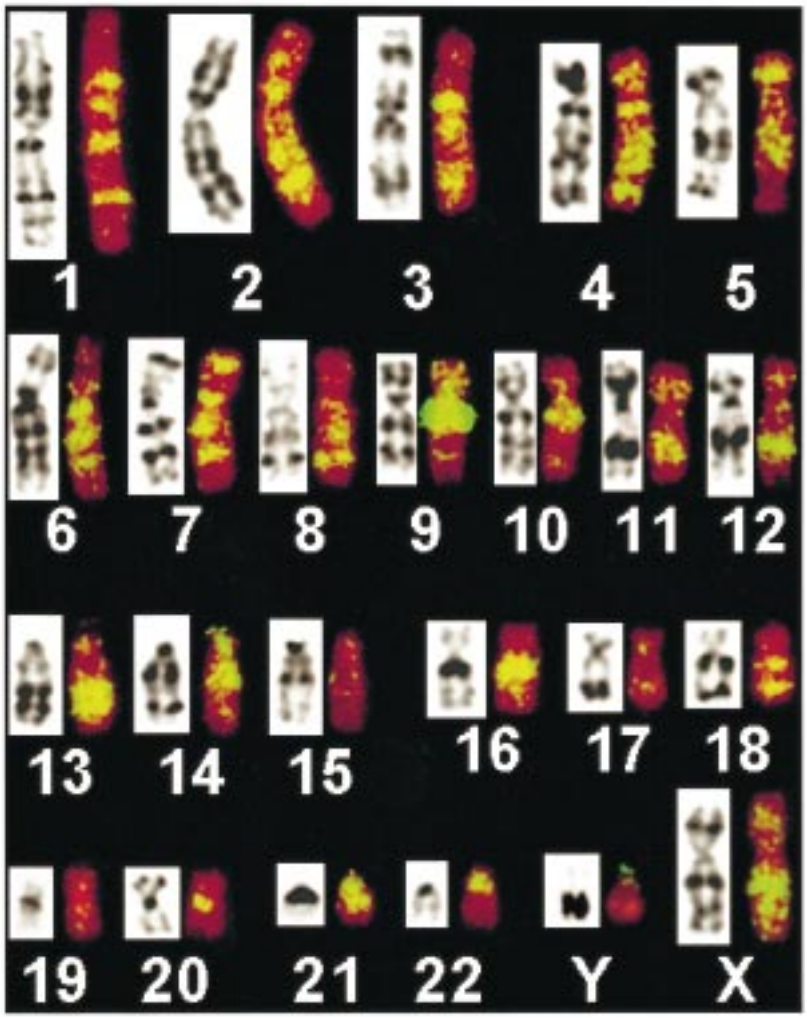


Figure 3. L1 isochore banding pattern. The left and right member of each chromosome pair shows the GTG bands and the L1 isochore hybridization, respectively. Biotinylated L1 isochores present in the pellet DNA shown in Figure 1 were detected with avidin–FITC. Chromosomes were stained with propidium iodide.

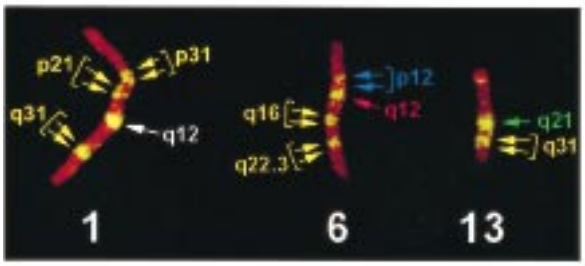


Figure 4. High-resolution localization of the L1 isochore family. Representative human chromosomes (1, 6 and 13) from Figure 2A showing high-resolution hybridization obtained with the pellet DNA. Coloured arrows indicate different types of hybridization. Yellow arrows: signals cover the corresponding G bands. Blue arrows: signals cover less than the corresponding G bands. Red arrow: signals cover more than the corresponding G bands. Green arrow: a single signal covers more than one G band. White arrow: hybridization on the pericentromeric heterochromatic band. This latter type of hybridization was also observed on chromosomes 9, 14, 16, 20, 21 and 22, indicating that the suppression of the repetitive sequences by unlabelled Cot1 DNA was insufficient in these regions.

This indicates that the level of DNA compaction in L1 bands is higher than the average (see Discussion).

The high-resolution hybridization obtained with pellet DNA gave rise to signals characterized by different features. The majority of L1 bands was present in doublets separated by thin non-hybridizing internal bands (see the yellow arrows in Figure 4 and Figure 5). The few exceptions are exemplified in the legend of Figure 4.

The G3 and G4 bands contain almost the totality of the GC-poorest isochores present in the pellet. More precisely, about 85% of the G4 bands and about 40% of the G3 bands showed strong signals, the remaining G3 and G4 bands showing very weak or no signals (see Figure 5). Only a few (about 4%) G2 bands and no G1 bands seem to contain L1 isochores. These are clear indications that G1 + G2 and G3 + G4 bands form two compositionally different subsets of G_{850} bands.

Finally, some G4 bands do not contain pellet DNA. These bands are 1q41, 2q36.3, 3p24.3, 4q34.3, 5q34, 8p22, 10q23.1, 10q25.1, 13q33.1, 13q33.3 and 14q12. It is possible that L1 isochores are also present in these G4 bands but in very small amounts. These bands appear to be a small distinct subset of G4 bands that do not replicate at the end of the S phase of the cell cycle (see below).

Interestingly, a non-overlapping localization of the GC-poorest and the GC-richest DNA of the human genome was evident when L1 and H3 isochores were hybridized simultaneously (Figure 6).

Chromosomal distribution of the GC-poor L2 isochores

The L2 isochores were present not only in fractions 1 and 2 but also in the pellet DNA (together with the L1 isochores) and in fraction 3 (together with H1 isochores) as shown in Figure 1. An analysis of the hybridization signals on at least five metaphases per fraction is summarized in Figure 1, which shows that L2 isochores are predominantly located on G bands, and, to a small extent, on $H3^-$ R bands, i.e. on the R bands that do not contain H3 isochores (Figure 2).

Replication timing of G bands

The correlation between the chromosomal bands that hybridized with the pellet and their replication timing (Dutrillaux *et al.* 1976) indicates that these bands replicate predominantly at the end of the S phase of the cell cycle (Figures 7 & 8). Indeed, all the bands that belong to classes XIV–XVIII of Dutrillaux *et al.* (1976) contain the GC-poor pellet DNA whereas only few bands belonging to classes X–XIII hybridize with the pellet.

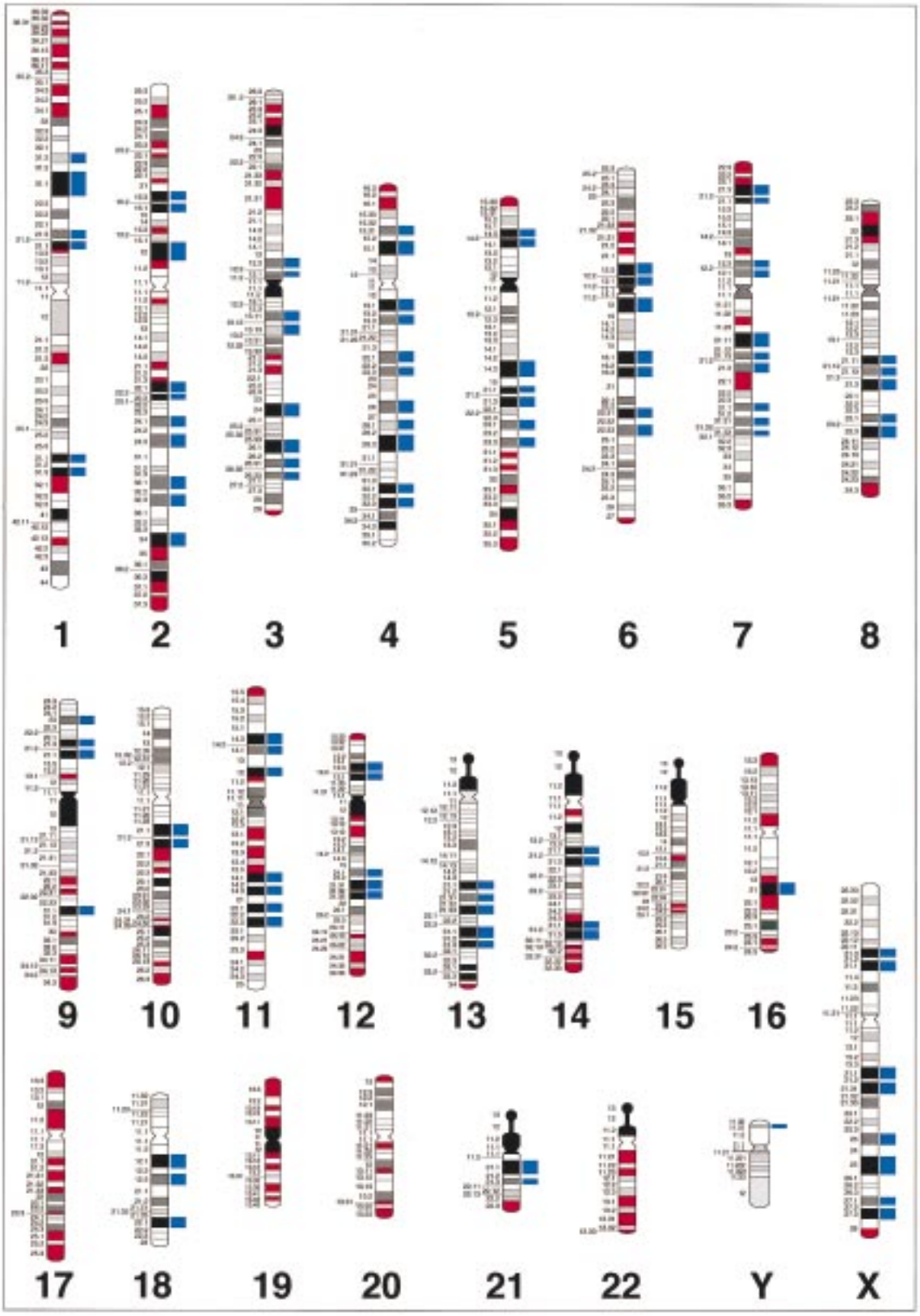
As described above, a number of G4 bands did not show detectable levels of hybridization with pellet DNA (see Figure 5). All these bands belong to classes X–XII, namely to those regions that replicate at the beginning of the second part of the S phase, in contrast to the majority of bands that hybridize with the pellet DNA. A possibility is that this set of bands was misclassified as G4 bands.

Discussion

The analysis of the GC levels of the G bands of human chromosomes, as obtained by *in-situ* hybridization of compositional fractions of human DNA, has led to the identification of two subsets of G bands, one of which is characterized by the lowest GC level and the latest replication. Thus, the GC-poorest regions of the human chromosomes are also the latest replicating regions, whereas the GC-richest bands are the earliest replicating regions (Federico *et al.* 1998).

Pellet DNA (about 40% of the total genomic DNA) is localized on the majority of G3 and G4 bands that correspond to about 26% of the karyotype, stressing the high DNA compaction

Figure 5. (opposite) Identification of the GC-poorest and the GC-richest chromosomal bands. Human karyotype at a resolution of 850 bands per haploid genome showing the chromosomal bands containing the GC-poorest (blue bars on the right of each chromosome) and the GC-richest isochores (red regions inside the chromosomes). The grey scale of the G bands is according to Francke (1994). The GC-richest isochore bands are from Saccone *et al.* (1999).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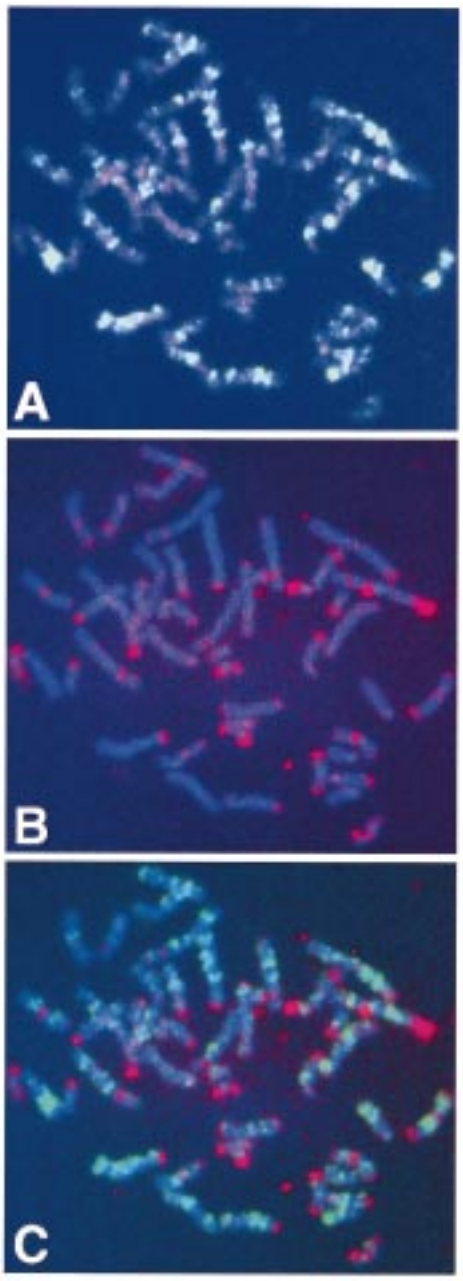

Figure 6. Colocalization of the L1 and H3 isochore families. Human metaphase simultaneously hybridized with (**A**) the digoxigenin-labelled GC-poorest DNA present in the pellet (see Figure 1), and (**B**) the biotin-labelled GC-richest DNA (see Materials and methods), detected by antidigoxigenin–FITC (green signals) and avidin–TRITC (red signals), respectively. (**C**) Merging of the hybridizations shown in **A** and **B**. Chromosomes were stained with DAPI.

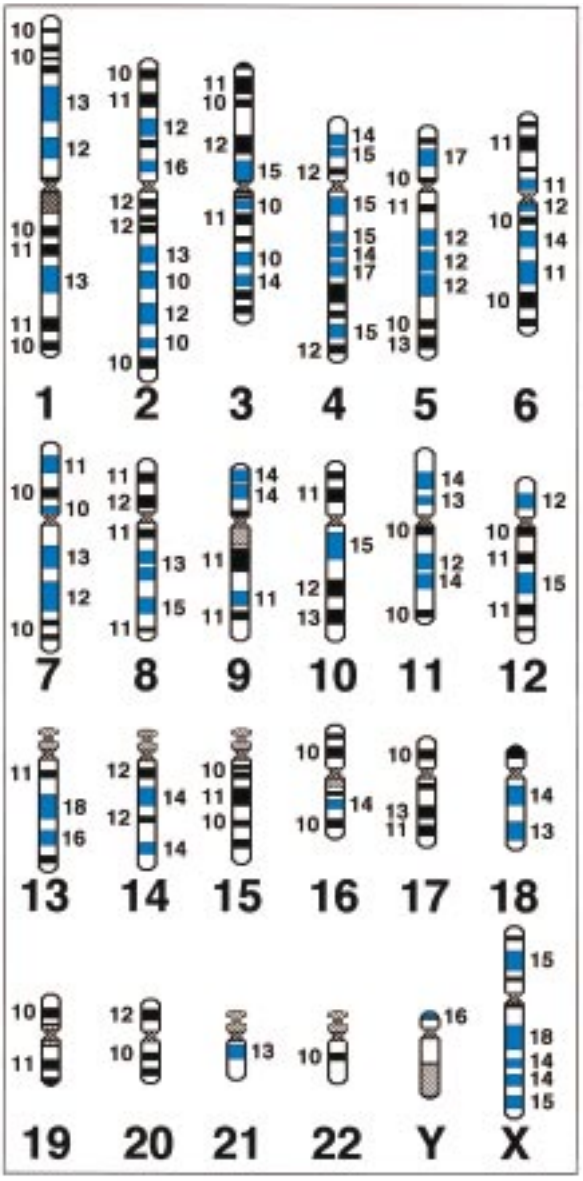

Figure 7. Replication timing of the G bands. Human karyotype at a resolution of 400 bands per haploid genome showing the chromosomal bands containing the very GC-poor isochores present in the pellet. Blue, black and white regions indicate the G bands containing the GC-poorest, L1, isochores, the other G bands, and the R bands, respectively. The numbers on the right and on the left of each chromosome indicate the replication timing of the corresponding regions, as described previously (Dutrillaux *et al.* 1976, Biemont *et al.* 1978).

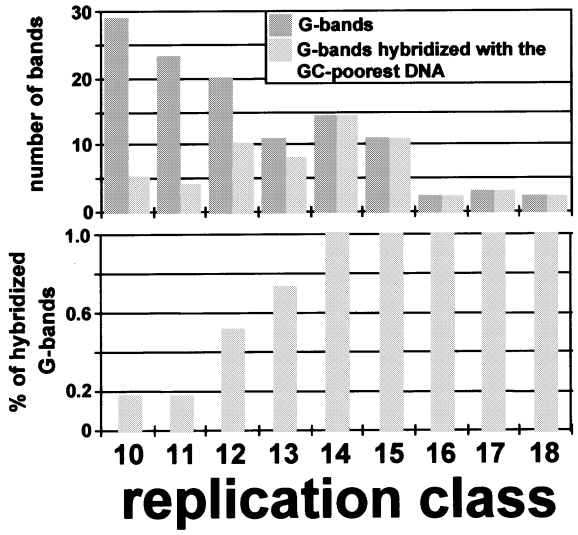

Figure 8. Replication timing of the GC-poorest chromosomal regions. Histograms showing the G bands hybridized with the pellet DNA as distributed in the replication classes described by Dutrillaux *et al.* (1976) and Biemont *et al.* (1978).

in those bands. In contrast, the H3 and H2 isochores (about 12% of the total genomic DNA) are located together on the $H3^{+}_{850}$ bands that represent about 17% in size of the total karyotype (Saccone *et al.* 1999). The remaining isochore families, L2 and H1, are prevalently distributed on the G1, G2 and $H3^{-}$ R bands.

The recent investigations on the nucleotide sequences of human chromosomes 22 and 21 (Dunham *et al.* 1999, Hattori *et al.* 2000) allow the analysis of the sequence features of their bands as far as isochore distribution and their correlation with the chromosomal bands are concerned. Moreover, an exact distribution of genes on the above chromosomes could be obtained. Our findings on the chromosomal distribution of the L1 and H3 isochores is confirmed: all the $H3^{+}$ bands of chromosomes 22 and 21 are largely composed of H3 and H2 isochores, and the only bands containing the L1 isochores are the G4 and G3 bands of chromosome 21. Moreover, gene concentration also defines the band type, being highest in the $H3^{+}$ bands and lowest in the L1 bands (Saccone *et al.* manuscript in preparation).

Our observations indicate that DNA located in G3 and G4 bands (that contain L1 isochores) is more compact than the average, whereas the opposite is true for DNA in $H3^{+}$ R bands (where H3 and H2 are located). At present, we cannot estimate the DNA compaction of G1, G2 and $H3^{-}$ R bands because the precise distribution of L2 and H1 isochores (which represent 30% and 24% of the genome, respectively) is difficult to define. The differential DNA compaction can be estimated for at least some bands located on chromosome 21 (Hattori *et al.* 2000). Indeed, there is about 20% more DNA than expected in the G4 band 21q21.1 and about 15% less DNA than expected in the $H3^{+}$ 21q22.3 band (Saccone *et al.* paper in preparation).

The different levels of grey indicated by Francke (1994) for the G bands of human chromosomes could, therefore, be due to a different composition of their DNA and/or to their different DNA compaction. In the past, a compositional homogeneity of the G bands was generally accepted, contrasting with the high heterogeneity of the R bands. The present results lead to the conclusion that even among G bands there is a compositional heterogeneity, the darker Giemsa bands being very GC-poor in contrast to the lighter G bands as well as being later replicating.

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