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Localization of the gene-richest and the gene-poorest isochores in the interphase nuclei of mammals and birds

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Abstract

At a resolution of 850 bands, human chromosomes comprise two subsets of bands, the GC-richest $H3^+$ and the GC-poorest $L1^+$ bands, accounting for about 17 and 26%, respectively, of all bands. The former are a subset of the R bands and the latter are a subset of the G bands. These bands showed the highest and the lowest gene densities, respectively, as well as a number of other distinct features. Here we report that human and chicken interphase nuclei are characterized by the following features. (1) The gene-richest/GC-richest chromosomal regions are predominantly distributed in internal locations, whereas the gene-poorest/GC-poorest DNA regions are close to the nuclear envelope. (2) The interphase chromosomes seem to be characterized by a polar arrangement, because the gene-richest/GC-richest bands and the gene-poorest/GC-poorest bands are predominantly located in the distal and proximal regions, respectively, of chromosomes, and because interphase chromosomes are extremely long. While this polar arrangement is evident in the larger chromosomes, it is not displayed by the chicken microchromosomes and by some small human chromosomes, namely by chromosomes that are almost only composed by GC-rich or by GC-poor DNA. (3) The gene-richest chromosomal regions display a much more spread-out conformation compared to the gene-poorest regions in human nuclei. This finding has interesting implications for the formation of GC-rich isochores of warm-blooded vertebrates.

Keywords: Chromosomal band; Isochore; Chromatin condensation; Chicken; Human; Nuclear architecture

1. Introduction

The genomes of warm-blooded vertebrates (neglecting satellite DNAs) cover a very broad compositional spectrum. Contrary to a view that was predominant until the early 1970s, the compositional heterogeneity of these genomes is not continuous but discontinuous (Filipski et al., 1973). Indeed, at fragment sizes of 50–100 kb, these DNAs are made up of a small number of families of molecules which are comparable in heterogeneity to the least heterogeneous natural DNAs, namely bacterial DNAs (Filipski et al., 1973; Cuny et al., 1981; see Bernardi, 2000 for a review). In turn, these families of DNA molecules derive, by degradation during DNA preparation, from much longer chromosomal regions (originally estimated as larger than 300 kb; Macaya et al., 1976) that were called isochores, for compositionally 'equal regions'. In other words, the genomes of warm-blooded vertebrates are mosaics of isochores. In the human genome, isochores belong to two GC-poor families, L1 and L2, and to three GC-rich families H1, H2 and H3 which represent about 33, 30, 24, 7.5 and 4–5%, respectively, of the genome (Bernardi et al., 1985; Mouchiroud et al., 1991; Zoubak et al., 1996). A similar situation was found in the chicken genome where, however, slightly higher GC values are attained by a very small isochore family, H4 (Cortadas et al., 1979; Olofsson and Bernardi, 1983; Kadi et al., 1993).

Gene densities are low in L1, L2 and H1 isochores, and high in H2 and H3 human isochores (Bernardi et al., 1985; Mouchiroud et al., 1991; Zoubak et al., 1996), a conclusion confirmed by recent work from other laboratories (Lander et al., 2001; Venter et al., 2001). Gene densities define two 'gene spaces' which have been called the 'empty quarter', representing about 85% of the genome, and the 'genome

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core', representing the remaining 15% or so of the genome (see Bernardi, 2000 for a review). A similar correlation between gene density and GC levels was found in chicken (Cacciò et al., 1994; Andreozzi et al., 2001). The genome core is not only characterized by a high gene density, but also by an early replication timing, a high recombination, an open chromatin structure, whereas the empty quarter is endowed with opposite properties.

In situ hybridization of human DNA fractions derived from different isochore families, under conditions of no interference by repeated sequences, allowed us to define two subsets of chromosomal bands at metaphase and prometaphase, namely at resolutions of 400 and 850 bands, respectively. The first subset is made up by the R(everse) bands that hybridize the gene-richest/GC-richest isochore family H3 (Saccone et al., 1992, 1996, 1999), the second subset by (G)iemsa bands that hybridize the gene-poorest/GC-poorest isochore family L1 (Federico et al., 2000). The former, $H3^+$, bands (which typically also comprise H2 isochores) represent 17% of all bands, whereas the latter, $L1^+$, bands represent 26% of all bands of the 850-band human karyotype (Francke, 1994). The remaining bands comprise the $H3^-$ R bands (36% of the total) and the $L1^-$ G bands (19% of the total), which do not hybridize H3 and L1 isochores, respectively. Interestingly, $H3^-$ and $L1^-$ bands can hardly be distinguished from each other on the basis of their GC levels, which are in the same intermediary range between $L1^+$ and $H3^+$ bands. $H3^-$ and $L1^-$ bands can, however, be distinguished on the basis of the GC levels of flanking bands which are GC-richer in the case of the $L1^-$ bands, and GC-poorer in the case of the $H3^-$ bands (Saccone et al., 2001). Two other points that should be mentioned are the lower compaction and the earlier replication of DNA of $H3^+$ bands compared to that of $L1^+$bands (Saccone et al., 1999, 2001; Federico et al., 1998, 2000).

In the present work, we investigated the distribution of the gene-richest and gene-poorest regions in human and avian interphase nuclei. We also studied the degree of 'openness' of chromatin in five human chromosomal regions characterized by different compositional properties.

2. Materials and methods

2.1. DNA probes

Compositional fractions of human and chicken genomic DNAs were obtained by preparative ultracentrifugation in Cs_2SO_4 density gradients in the presence of the sequence-specific DNA ligand 3,6-bis(acetato-mercuri-methyl)dioxane, BAMD, as previously described (Cuny et al., 1981). The GC-poorest and the GC-richest fractions of human and chicken genomes were then separated (see Andreozzi et al., 2001; Saccone et al., 1996), characterized in their CsCl profiles and modal buoyant densities, ρ_0, and labeled with biotin or digoxigenin using a nick translation kit (Roche, Germany).

Some band- and chromosome-specific DNA probes were also used. The biotin-labeled human DNA from bands 6p21, 9p24, 9q34.2–34.3, 12q21, 12q22–23 were from LiStar FISH (Italy). The digoxigenin-labeled DNAs for the painting of chromosomes 6, 9 and 12 were obtained from Oncor (UK).

2.2. Preparation of chromosomes and nuclei

Human metaphase chromosomes and nuclei were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes by standard cytogenetic procedures. Metaphase and interphase cells were obtained from chicken primary fibroblast lines as already described (Andreozzi et al., 2001). Conventional methanol/acetic acid fixation and slide preparation were then carried out, since no differences in the final results were observed when special procedures for preserving cell integrity (Croft et al., 1999; Bridger et al., 2000) were used.

2.3. In situ hybridization and detection

Two hundred nanograms of the biotin labeled GC-richest and 200 ng of the digoxigenin-labeled GC-poorest DNA fractions were hybridized as previously described (Saccone et al., 1999; Federico et al., 2000; Andreozzi et al., 2001). The biotin-labeled DNAs from human chromosomal bands were co-hybridized with chromosome-specific DNA using the conditions indicated by the manufacturers. Avidin conjugated with tetramethylrhodamine isothiocyanate (TRITC-avidin) and anti-digoxigenin antibody conjugated with fluorescein isothiocyanate (FITC-anti-digoxigenin antibody) were used for detection.

3. Results

3.1. Distribution of GC-richest and GC-poorest isochores in prometaphase chromosomes

The GC-poorest fractions (corresponding to the pellet DNA) from both human and chicken genomes were mostly composed of L1 isochores ($\rho_0 = 1.6968$ g/cm^3 in human DNA; $\rho_0 = 1.6978$ g/cm^3 in chicken DNA; see Federico et al., 2000; Andreozzi et al., 2001), whereas the GC-richest fractions only contained human H3 ($\rho_0 = 1.7136$ g/cm^3) or chicken H4 ($\rho_0 = 1.7190$ g/cm^3) isochores (see Saccone et al., 1996; Andreozzi et al., 2001). As expected, these DNA fractions hybridized on the two sub-sets of the human and chicken chromosomal bands previously described (Saccone et al., 1999; Federico et al., 2000; Andreozzi et al., 2001), thus highlighting the GC-richest and the GC-poorest chromosomal regions. These two subsets of the human chromosomal bands are shown in Fig. 1, where the

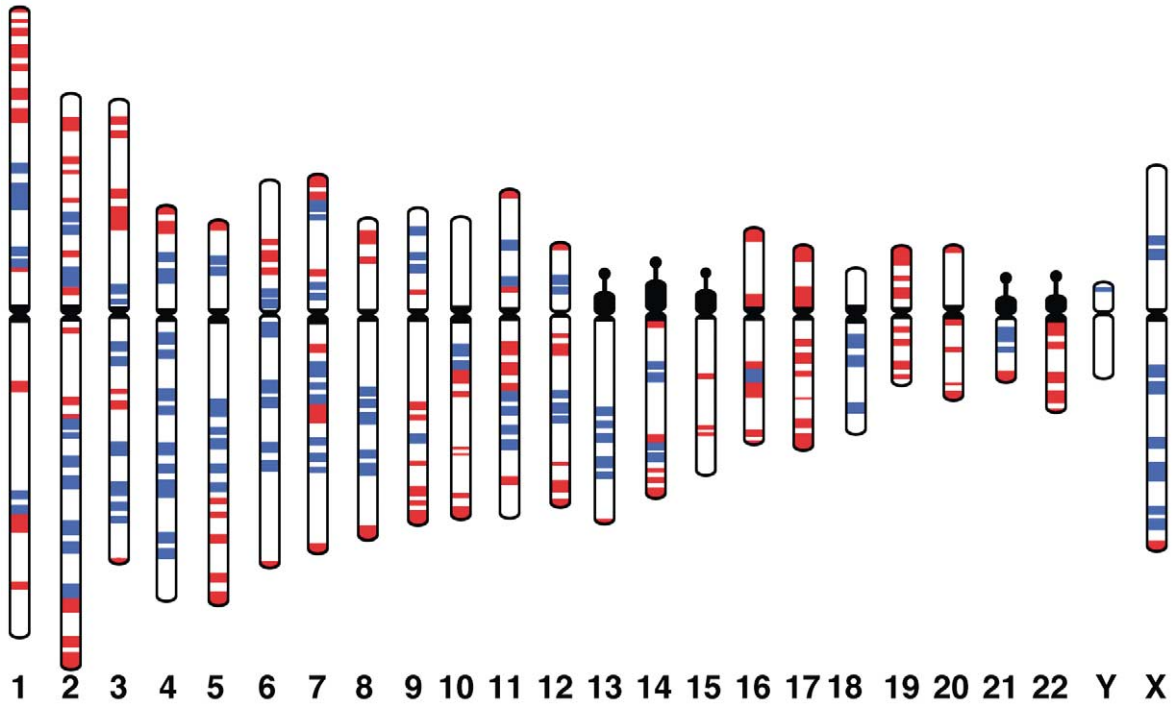

Fig. 1. Chromosome distribution of the $H3^+$ and $L1^+$ bands. Ideogram of human chromosomes at 850-band resolution showing the localization of the $L1^+$ (blue) and the $H3^+$ (red) bands as previously identified on the basis of the concentration of L1 and H3 isochores, respectively (Saccone et al., 1999; Federico et al., 2000). This figure shows that the gene-richest, GC-richest, $H3^+$ bands are prevalently located distally on each chromosome arm and are generally not adjacent to the gene-poorest, GC-poorest, bands, which are prevalently located more proximally. The intermediate bands ($H3^-$, $L1^-$) are left uncolored, to emphasize this spacing.

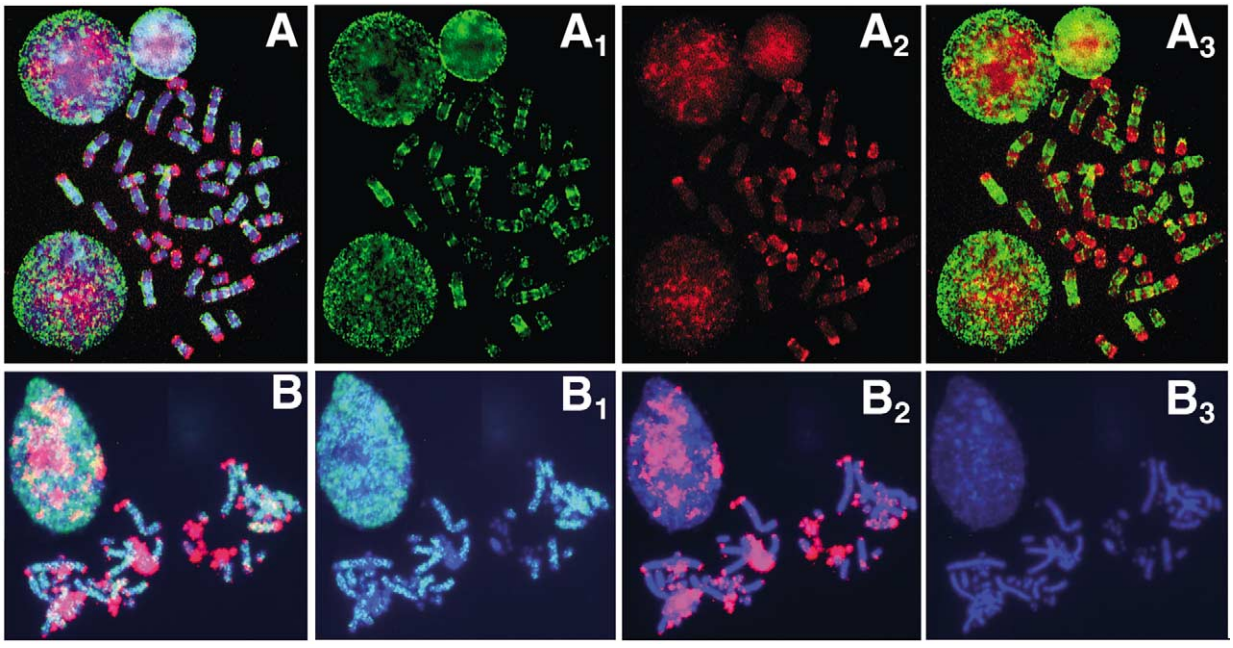

Fig. 2. Distribution of the GC-poorest and GC-richest isochores in human and chicken nuclei. Human (A) and chicken (B) chromosomes and nuclei hybridized with the GC-poorest L1 and the GC-richest H3 (in human) or H4 (in chicken) isochores. The H3 (or H4) and the L1 isochores were labeled with biotin and digoxigenin, respectively, and detected with TRITC-avidin (red signals) and FITC-anti-digoxigenin (green signals), respectively. The yellow color is due to the overlapping of red and green signals. Nuclei and chromosomes were stained with DAPI (blue). The images were obtained with epifluorescence microscopes equipped with the appropriate filters, and captured with a CCD camera. MacProbe 4.2.3 and Photoshop 5.0 software were used to obtain the images. (A_1), (A_2), and (A_3) are as in (A), without the DAPI stain, showing the green, the red, and both the green and red signals, respectively. (B_1), (B_2), and (B_3) are as in (B) showing the green signals, the red signals, and the DAPI stain, respectively.

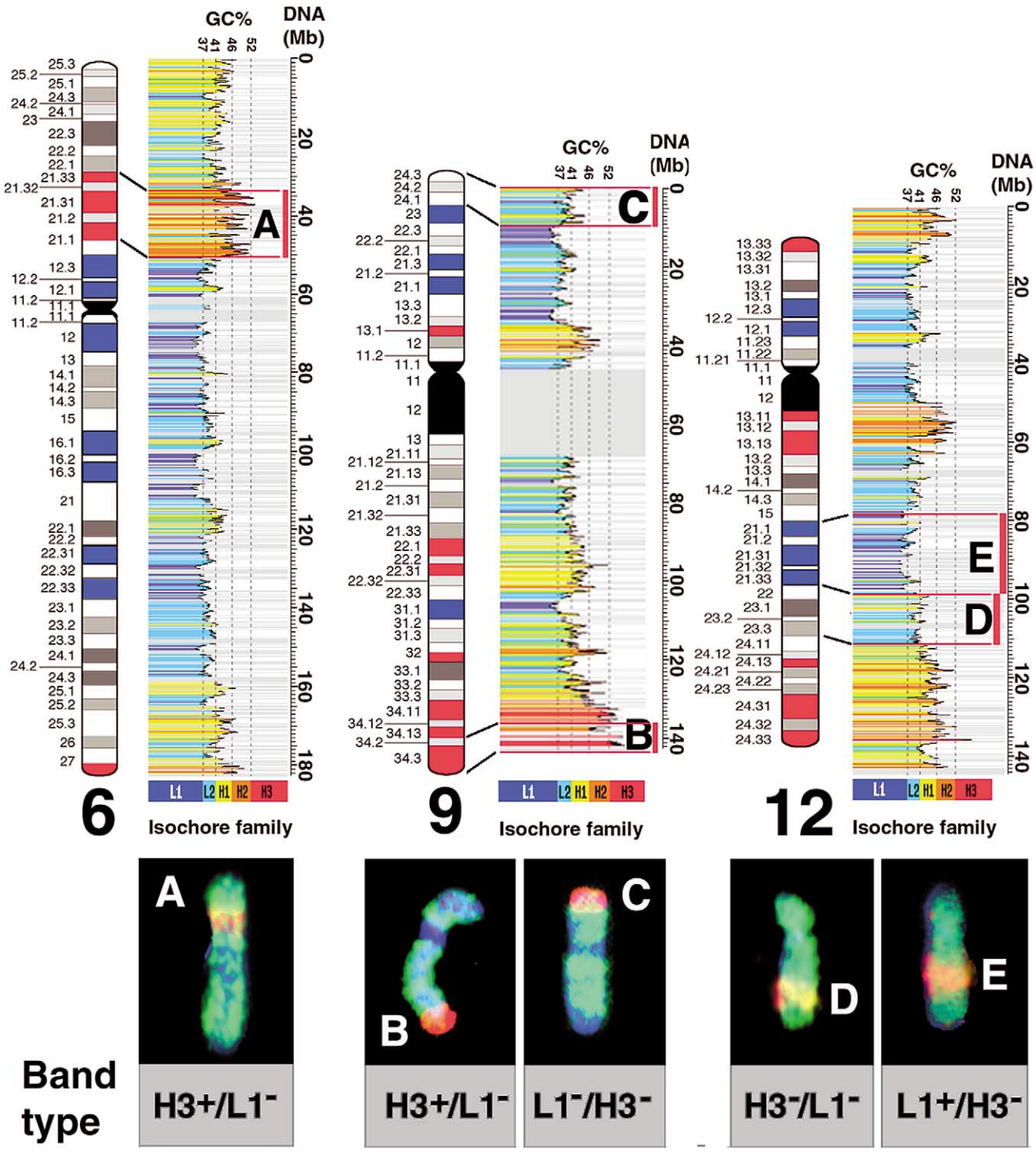

Fig. 3. Band DNA probes used to demonstrate the differential chromatin compaction. (Top) Ideograms of human chromosomes 6, 9, and 12 at 850 band resolution (Francke, 1994), and corresponding GC profiles. The ideograms, show the $H3^+$ bands (Saccone et al., 1999) in red and the $L1^+$ bands (Federico et al., 2000) in blue. The GC profiles (from Pavliček et al., 2002) shown on the right of each chromosome ideogram are differentially colored according to the GC level of the isochore families (L1, blue: < 37%; L2, pale blue: 37–41%; H1, yellow: 41–46%; H2, orange: 46–52%; H3, red: > 52%). The chromosomal bands were matched with the GC profiles according to Saccone et al. (2001). A, B, C, D, and E indicate the probes for the in situ hybridizations shown in Fig. 4. The gray regions in the GC profiles are unsequenced regions. (Bottom) Chromosomes 6, 9 and 12 showing the hybridization with the biotin-labeled probes A–E. Chromosomes were painted with digoxigenin-labeled probes. Detection was performed as in Fig. 2. Band type indicates the compositional features of the hybridized bands (at a resolution of 850 bands). Probes A and B detected regions mostly consisting of the GC-richest $H3^+$ bands; probes C and D detected regions composed by GC intermediate $L1^-$ and $H3^-$ bands, but adjacent to GC-poorest bands; probe E detected regions mostly consisting of the GC-poorest $L1^+$ bands.

general non-contiguity of the $L1^+$ and $H3^+$ bands is emphasized by leaving uncolored the intermediate bands shown in the original figure (Fig. 5 from Federico et al., 2000). The $H3^+$ bands are largely located in the centromeric-distal regions and the $L1^+$ bands in the centromeric-proximal regions of chromosomes.

3.2. Distribution of GC-richest and GC-poorest isochores in the interphase nuclei of human and chicken

The in situ hybridization of the human GC-richest fraction showed a general distribution of the signals in the central region of the nuclei, whereas the GC-poorest fraction was detected prevalently in the peripheral part of the nuclei (Fig. 2A), all the observed nuclei showing this type of signal distribution. Also the hybridization of the chicken GC-richest and the GC-poorest DNAs indicated that the GC-richest regions are localized in the interior of the nucleus, whereas the GC-poorest ones are localized in the peripheral part (Fig. 2B). The chicken nuclei also showed some large H4-hybridizing peripheral regions that could be due to the heterochromatin of the Z chromosome (macrosatellite pFN-1; see Hori et al., 1996), which was incompletely competed out by the suppression procedure (see Andreozzi et al., 2001).

3.3. Different compaction of the human GC-richest and GC-poorest chromosomal regions in interphase nuclei

To better understand, at the nuclear level, the chromatin conformation as related to the GC levels of the bands, we investigated in detail (see Fig. 3) the GC-richest bands 6p21 (probe A) and 9qter (probe B), the GC-intermediate bands 9pter (probe C) and 12q22-23 (probe D), and the GC-poorest band 12q21 (probe E). The compositional features of each band were obtained from previous chromosomal compositional map data (Saccone et al., 1996, 1999; Federico et al., 2000). The GC-profiles shown in Fig. 3 were obtained using the draft human genome sequence (Lander et al., 2001), as redrawn in Pavlíček et al. (2002), and correlated to chromosomal bands as recently described (Saccone et al., 2001). The band DNA probes, together with the corresponding chromosome-specific DNA, were first hybridized on metaphase chromosomes to check the precise localization of the probes in the chromosomes (see Fig. 3) and to show that no cross-hybridization on other chromosomal sites could be detected with any of the five probes.

Each of the five chromosomal regions (Fig. 3, probes A–E) were then hybridized on the cell nuclei (Fig. 4A–E) and the extension of the hybridization signals in mitotic (Fig. 3) and interphase (Fig. 4) cells was analysed. The GC-rich 6p21 and 9qter regions (Fig. 3A,B) showed a wider spreading of the hybridization signals in the nuclei (Fig. 4A, B) relative to that of the chromosome territories. Indeed, a comparison of the same hybridization on metaphase (Fig. 3, probes A and B) and interphase (Fig. 4A,B) chromosomes showed that the extensions of the two band DNAs in the nuclei are clearly more spread out than those of mitotic chromosomes. In contrast, the GC-poor 12q21 region (Fig. 3, probe E) showed a high level of compaction in the nuclei (Fig. 4E). In fact, the comparison of the hybridizations in the metaphase (Fig. 3, probe E) and interphase (Fig. 4E) chromosomes clearly indicated that hybridization signals of the band-specific DNA are more condensed in the nuclei compared to the mitotic cells. The hybridization obtained with the GC-rich and the GC-poor probes (Fig. 4) were detected on about 80–85% of the nuclei.

The compositionally intermediate region 9pter (comprising $H3^-$ and $L1^-$ bands) showed a compaction degree comparable to that observed with the GC-poor band 12q21, the large majority of the nuclei exhibiting the hybridization

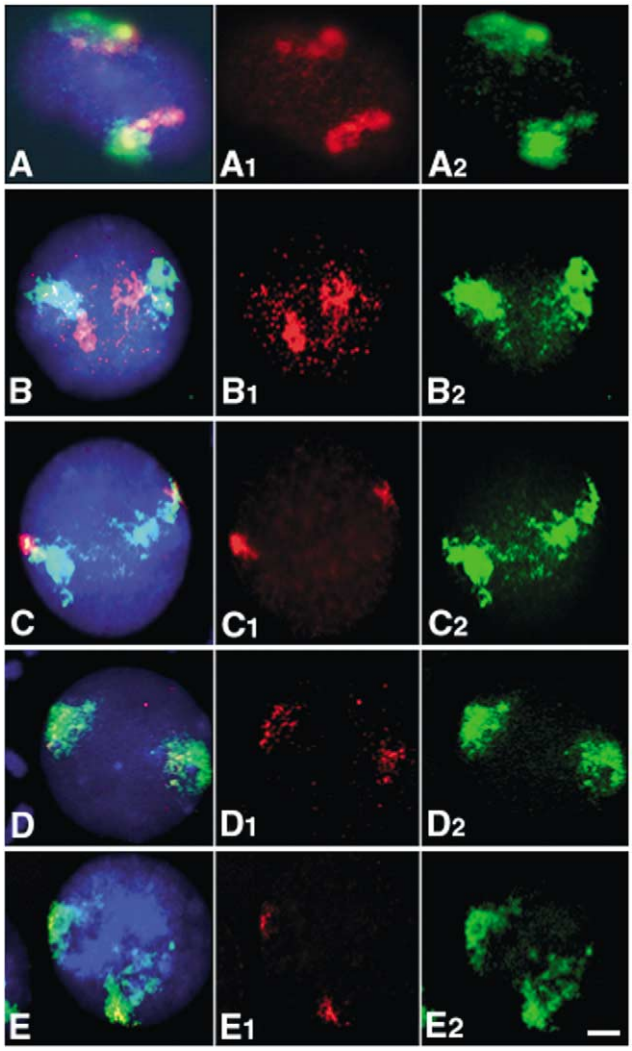

Fig. 4. Nuclear distribution of chromosomal regions characterized by different GC levels. Chromosomal regions corresponding to bands 6p21 (A), 9q34.2–34.3 (B), 9p24 (C), 12q22–23 (D), and 12q21 (E) were co-hybridized with the corresponding chromosome probes. Band and chromosome DNAs were biotin- and digoxigenin-labeled, respectively. Detection was done as in Fig. 2. Bar: 2 μm.

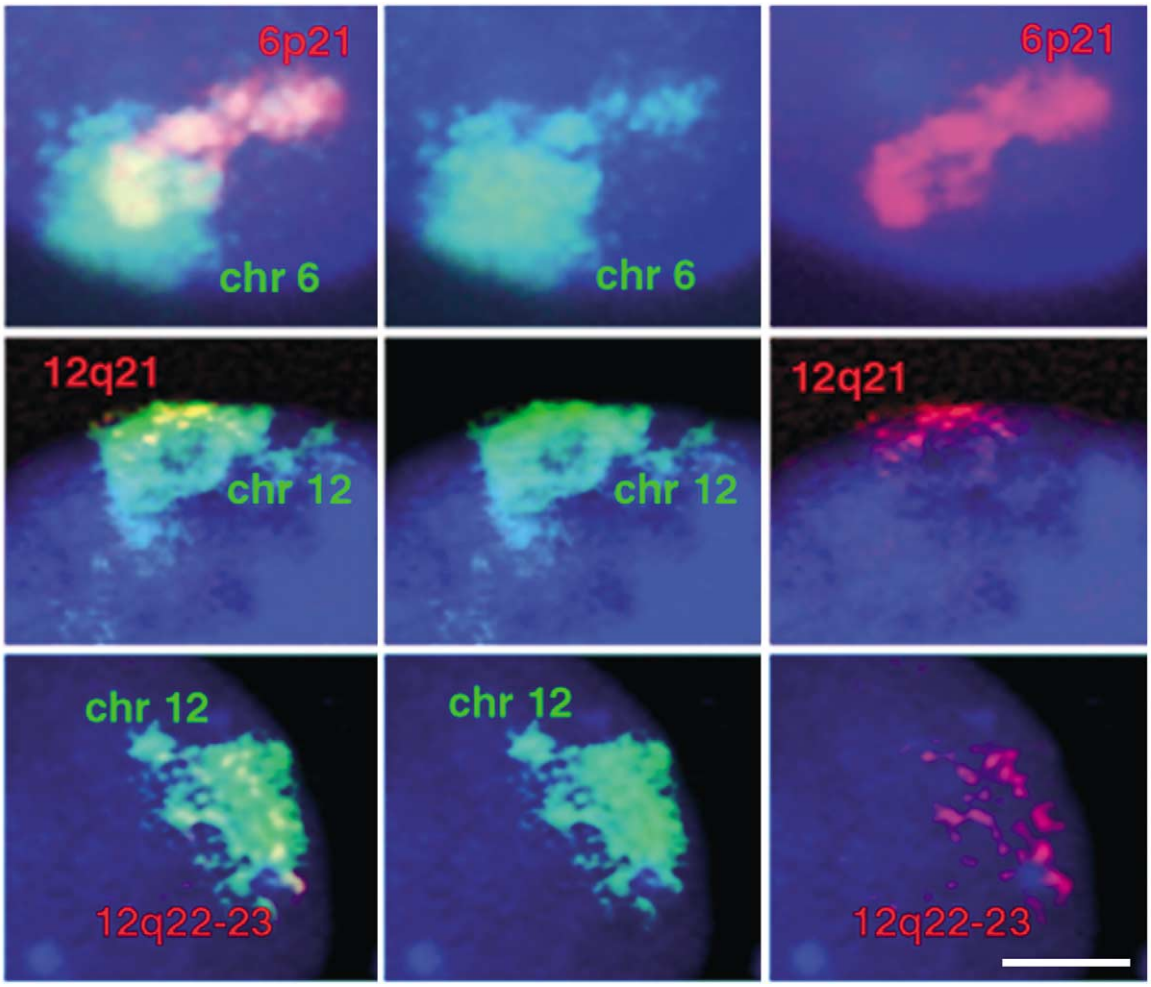

Fig. 5. Differential compaction of chromatin in human nuclei. Comparison of hybridizations obtained with the GC-richest and the GC-poorest DNA regions (partial nuclei from Fig. 4). The band DNA (red signals) and the corresponding chromosome (green signals) are indicated. Nuclei were DAPI stained (blue). The very GC-rich 6p21 band showed a much larger extension compared to the very GC-poor 12q21. Furthermore, the former is more internal compared to the latter. The results obtained with the intermediary band 12q 22–23 are also shown for the sake of comparison. Bar: 2 μm.

signal distribution of Fig. 4C. Instead, the other GC-intermediate band, 12q22–23 (Fig. 3, probe D), showed a signal distribution in the nuclei (Fig. 4D) characterized by features intermediate between those of the GC-rich and GC-poor bands, although a number of nuclei (about 25%) were observed with more compact signals, similar to those of the GC-poor band 12q21. In any case, the two GC-intermediate bands tested never showed signals similar to those obtained for the GC-rich bands. It should be noted that the two GC-intermediate regions are located in close contiguity to bands of the GC-poorest class, suggesting that GC-intermediate bands are characterized by compaction features similar to those of the adjacent bands.

The different compaction level of the GC-rich and the GC-poor chromosomal regions is also evident by comparing their hybridizations in the nuclei. In fact, in spite of the fact that the GC-rich 6p21 and the GC-poor 12q21 bands comprise a similar DNA amount (15–20 Mb; see Fig. 3, probes A and E), the spreading of the signals in the nuclei is wider for the former compared to the latter (see Fig. 5).

Concerning the nuclear location of the DNA belonging to bands endowed with different compositional features, the results are in agreement with the nuclear distribution of the GC-richest and the GC-poorest isochores (Fig. 2). In fact, we observed a more peripheral localization of the GC-poor bands compared to a more internal position of the GC-rich bands (see Fig. 5). In the case of chromosome 9, this indicates that different regions of the same chromosome contribute to the DNA located both in the interior and the periphery of the nucleus. Moreover, at a resolution of 850 bands (see Fig. 3), probes A and B (from two GC-richest chromosomal regions) and probe E (from one of the GC-poorest regions), detected not only the $H3^+$ and the $L1^+$ bands, respectively, but also the $L1^-$and $H3^-$ the bands, respectively, that are located between them. This indicates that DNA from these very small compositionally inter-

mediate $L1^-$ and $H3^-$ bands also occupy the same nuclear location as the contiguous GC-richest or GC-poorest bands.

4. Discussion

4.1. Distribution of the GC-richest and the GC-poorest DNAs

We have shown that the GC-richest and the GC-poorest DNA of warm blooded vertebrates, and more precisely DNA characterized by GC levels higher than 50% (H3 isochore family) and lower than 38% (L1 isochore family), are located in internal and peripheral regions, respectively, of the nucleus. No statistical evaluation of the signal distribution (after dividing the nuclei in concentric shells, as previously reported by other researchers) was necessary, the H3 and L1 signals being clearly located in internal and peripheral location, respectively, as shown in Fig. 2. Interestingly, a contrasting location was previously observed at the chromosomal level (see Fig. 1), the GC-richest regions being largely located in telomeric position and the GC-poorest regions more internally in the chromosomes. The importance of this observation is due to the fact that a number of structural and functional features of the genomes of warm-blooded vertebrates, in particular gene density, are correlated with GC levels (see Introduction).

The DNA probes used in the present work, namely the H3 and L1 isochores, detect two subsets of chromosomal bands, namely the $H3^+$ and the $L1^+$ bands, which largely overlap with the T bands of Dutrillaux (1973) and with the two darkest sets of G bands of Francke (1994), respectively. However, in the former case, this correspondence is only a general feature, since our $H3^+$ bands were detected at a 850-band resolution, whereas the T bands of Dutrillaux were obtained at a low resolution (~300 bands) and were very heterogeneous, being formed by sub-bands characterized by different GC-level and gene-density (Saccone et al., 1996, 1999). It should be stressed (1) that $H3^+$ and $L1^+$ bands correspond to the highest and lowest gene density bands at a resolution of 850 bands, whereas other investigations (Croft et al., 1999) concerned the totality of R bands and G bands at the 400-band resolution; (2) that, in the past, R bands were assumed to be GC-rich and gene-rich and, conversely, G-bands were assumed to be GC-poor and gene-poor. As far as the latter point is concerned, it should be pointed out that this view is incorrect, a number of G bands being characterized by a gene density comparable to that of some R bands. Moreover, not all G bands are characterized by a GC-level lower than the R bands (Saccone et al., 2001). Indeed, G-bands and R-bands exist which are endowed with

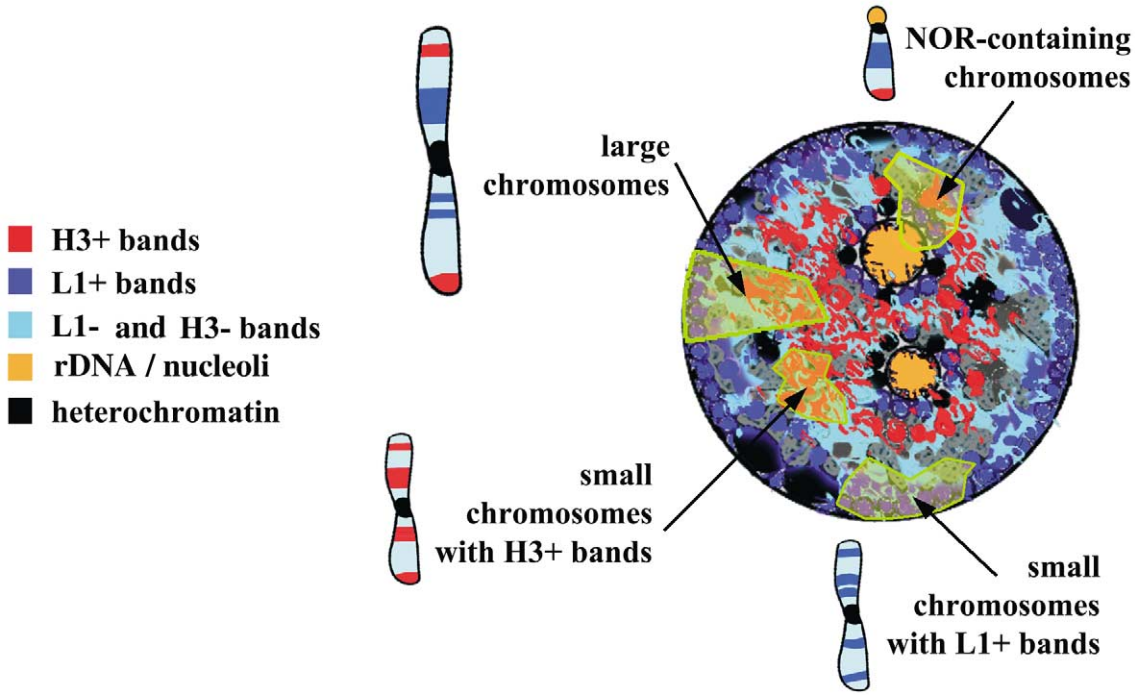

Fig. 6. Chromosome polarity in the nucleus. This scheme (modified from Sadoni et al., 1999) shows the different distribution of the chromosomes in the nuclei in relationship to their isochore content. The L1 isochores (present in the $L1^+$ bands) are distributed in the periphery of the nucleus, whereas the H3/H2 isochores (present in the $H3^+$ bands) are located in the interior. Since $L1^+$ and $H3^+$ bands are almost never contiguous in the mitotic chromosomes (Federico et al., 2000), a polar arrangement could be displayed in the nucleus. In fact, large chromosomes containing both $L1^+$ and $H3^+$ could spread from the periphery to the interior of the nuclei with the intermediate bands ($H3^-$ and $L1^-$, belonging to the R and G bands, respectively) distributed in all the nuclear compartments, namely in the regions defined by the contiguous $H3^+$ or $L1^+$ bands. This scheme also explains the more peripheral location of chromosome 18 (a small chromosome not containing $H3^+$ bands) and the more interior location of chromosome 19 (a small chromosome not containing $L1^+$ bands). The NOR-containing chromosome long arms could be located in the periphery of the nucleoli, being the rDNA and the centromeric heterochromatin in close proximity.

similar compositional features that are very different from those of $H3^+$ and $L1^+$ bands. These intermediate bands, called $L1^-$ and $H3^-$ bands, belonged to the G bands and to the R bands respectively.

The GC intermediate band DNAs ($L1^-$ and $H3^-$) are characterized by a nuclear location similar to those of the $L1^+$ or $H3^+$ bands that are close to them on mitotic chromosomes. In other words, the $L1^-$ and $H3^-$ bands have a widespread distribution in the nucleus, being present in both the internal and the peripheral nuclear regions.

4.2. Compositional polarity of the chromosomes

The large majority of chromosomes comprise both categories of compositionally very distinct bands, namely the $H3^+$ and the $L1^+$ bands (Federico et al., 2000; see also Fig. 1). It is, therefore, reasonable to assume that most chromosomes contribute to both the DNA located in the internal and peripheral parts of the nuclei. This is possible because the GC-richest and the GC-poorest bands are largely located distally and proximally, respectively (and are, therefore, only rarely adjacent in human chromosomes; see Fig. 1), and because of the large extension of interphase chromosomes. The observation that many chromosomes, especially the large ones, are spread in the nucleus from the periphery to the interior, even if in different proportions (Boyle et al., 2001), supports our proposal about the compositional polarity of the interphase chromosomes. This is shown in Fig. 6, which is slightly different from the original figure (Sadoni et al., 1999) where the chromosomes

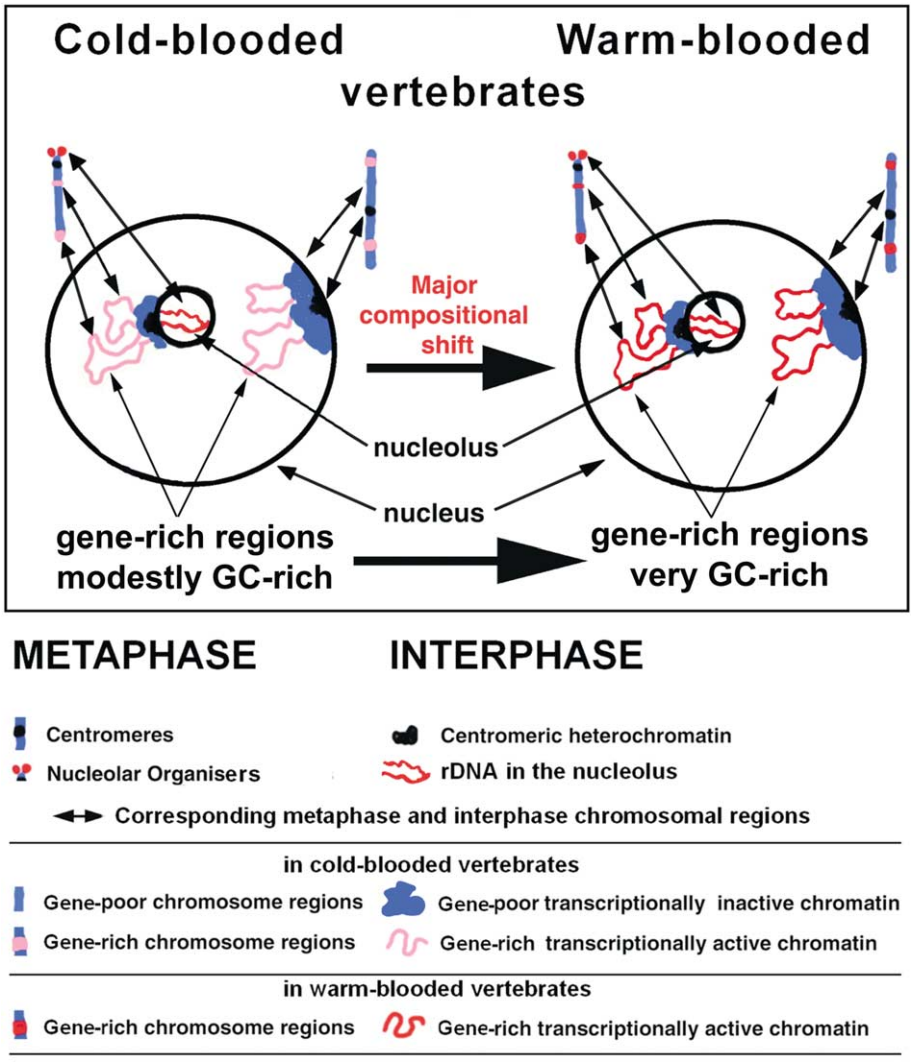

Fig. 7. The major compositional transition and the GC-rich isochore formation. Three chromosomes were represented in their mitotic and interphase configurations. In the warm-blooded vertebrates, the chromosomal regions with the highest concentration of genes are colored in red (representation of the $H3^+$ bands, the 'genome core'). In the nucleus, these regions are more open respect to the remaining gene-poor regions ('empty quarter'). If we consider that this could be the situation also in the cold-blooded vertebrates, thus the GC-rich isochores should be originated in the 'genome core' to enhance its thermal stability, being the chromatin highly decondensed. This stabilization was not needed either in cold-blooded vertebrates, because of their lower body temperature, nor in the 'empty quarter' of warm-blooded vertebrates, where the stability is provided by the compact chromatin structure itself. This could explain why the compositional changes were regional, instead of concerning the totality of the genome.

are considered to be composed by the classical R and G bands. It should be noted that the nuclear distribution of the human H3 isochores was, in fact, previously described to spread all over the interior of the nucleus (Sadoni et al., 1999), but in that case the hybridization was considered as due to the totality of the R bands and not to a sub-set of them, as shown here.

Our proposal accounts for the fact that chromosome 18 (containing $L1^+$, $L1^-$ and $H3^-$ bands, but no $H3^+$ bands) shows a peripheral nuclear localization, whereas chromosome 19 (which contains $H3^+$, $L1^-$ and $H3^-$ bands, but no $L1^+$ bands) is located in the nuclear interior (Croft et al., 1999). In other words, these exceptional situations are due to the fact that these two very small chromosomes lack the GC-richest and the GC-poorest bands, respectively (see Fig. 6). In fact, the only chromosomes showing a more internal location are chromosomes 19, 22 and 16 (Boyle et al., 2001), namely the small chromosomes containing mostly GC-richest isochores.

Basically, the chicken genome showed the same compositional properties observed for the human genome, both at the chromosomal and the nuclear level, the only difference being the large number of GC-richest bands that are present on microchromosomes (Andreozzi et al., 2001; see Fig. 2B). In chicken, the large majority of microchromosome DNA only contributes to the internal part of the nucleus, like the small GC-richest human chromosomes, whereas the large chromosomes, comprising both GC-richest and GC-poorest bands, contribute to both the interior and the periphery of the nucleus.

4.3. Differential compaction of DNA belonging to $H3^+$ and $L1^+$ bands

We showed that the two subsets of bands investigated here not only have different locations in the nucleus, but also correspond to different chromatin conformations, the $H3^+$ band DNA being remarkably more relaxed compared to the $L1^+$ band DNA. In this case, no statistical evaluation was done in order to estimate the rate of decondensation because the different compaction of the different bands was very evident. These results are understandable if we consider the special properties of H3 isochores compared to L1 isochores (see Section 1). In fact, the more open chromatin of the $H3^+$ band DNA corresponds to the highest concentration of genes and to the highest level of transcriptional activity. It should be noted, however, that our results are in general agreement with other data obtained with different methods showing that chromatin belonging to G and R bands of mitotic chromosomes is more dense and more open, respectively (Croft et al., 1999; Yokota et al., 1997), and that highly expressed sequences extend outside the chromosome territory (Volpi et al., 2000). Incidentally, the latter observation concerned a chromosomal region, 6p21, which was also investigated here. Also in these cases, the previous results concerned R and G bands (see above), and the subsets of bands investigated here.

4.4. The origin of the GC-rich isochores

In conclusion, the two 'gene spaces' previously described, the gene-rich 'genome core' and the gene-poor 'empty quarter', correspond to the genome compartments located in the interior and at the periphery of the nucleus, respectively. This supports a functional compartmentalization of the nucleus related to nuclear architecture (Strouboulis and Wolffe, 1996). The functional compartmentalization of the cell nucleus has also been demonstrated by other findings, such as the different replication timing of DNA located in the internal and peripheral part of the nucleus (Sadoni et al., 1999; Ferreira et al., 1997). This is in agreement with our present findings, since we previously demonstrated that the GC-richest $H3^+$ band DNA is replicated at the onset of the S phase whereas the GC-poorest $L1^+$ band DNA is replicated at the end (Federico et al., 1998, 2000).

The different spatial distribution of genes in the nucleus and the different chromatin compaction are apparently general for all vertebrates (work in progress), as expected from previous comparative investigations (see Bernardi, 2000 for a review). The different chromatin compaction of isochores belonging to different families in interphase nuclei suggests a model for the compositional transition from the 'ancestral', moderately GC-rich, genome core of cold-blooded vertebrates to the very GC-rich genome core of warm-blooded vertebrates. Indeed, as body temperature increased with the appearance of homeothermy in ancestral mammals and birds, the DNA located in the very open chromatin may have needed a GC increase to be stabilized, whereas this increase was not needed for the DNA, which was already stabilized by the closed chromatin structure (Fig. 7). This working hypothesis, to be presented in more detail elsewhere, would account for two important features of the formation of GC-rich isochores, namely the fact that only the gene-rich minority of the genome, the genome core, underwent the change and the fact that the change affected both coding and non-coding sequences of the genome core.

References

Andreozzi, L., Federico, C., Motta, S., Saccone, S., Sazanova, A., Sazanov, A., Smirnov, A., Galkina, S.A., Lukina, N.A., Rodionov, A.V., Carels, N., Bernardi, G., 2001. Compositional mapping of chicken chromosomes and identification of the gene-richest regions. Chromosome Res. 9, 521–532.

Bernardi, G., 2000. Isochores and the evolutionary genomics of vertebrates. Gene 241, 3–17.

Bernardi, G., Olofsson, B., Filipski, J., Zerial, M., Salinas, J., Cuny, G., Meunier-Rotival, M., Rodier, F., 1985. The mosaic genome of warm-blooded vertebrates. Science 228, 953–958.

Boyle, S., Gilchrist, S., Bridger, J.M., Mahy, N.L., Ellis, J.A., Bickmore, W.A., 2001. The spatial organization of human chromosomes within

the nuclei of normal and emerin-mutant cells. Hum. Mol. Genet. 10, 211–219.

Bridger, J.M., Boyle, S., Kill, I.R., Bickmore, W.A., 2000. Re-modelling of nuclear architecture in quiescent and senescent human fibroblasts. Curr. Biol. 10, 149–152.

Cacciò, S., Perani, P., Saccone, S., Kadi, F., Bernardi, G., 1994. Single-copy sequence homology among the GC-richest isochores of the genomes from warm-blooded vertebrates. J. Mol. Evol. 39, 331–339.

Cortadas, J., Olofsson, B., Meunier-Rotival, M., Macaya, G., Bernardi, G., 1979. The DNA components of the chicken genome. Eur. J. Biochem. 99, 179–186.

Croft, J.A., Bridger, J.M., Boyle, S., Perry, P., Teague, P., Bickmore, W.A., 1999. Differences in the localization and morphology of chromosomes in the human nucleus. J. Cell Biol. 145, 1119–1131.

Cuny, G., Soriano, P., Macaya, G., Bernardi, G., 1981. The major components of the mouse and human genomes. 1. Preparation, basic properties and compositional heterogeneity. Eur. J. Biochem. 115, 227–233.

Dutrillaux, B., 1973. Nouveau système de marquage chromosomique: les bandes T. Chromosoma 41, 395–402.

Federico, C., Saccone, S., Bernardi, G., 1998. The gene-richest bands of human chromosomes replicate at the onset of the S-phase. Cytogenet. Cell Genet. 80, 83–88.

Federico, C., Andreozzi, L., Saccone, S., Bernardi, G., 2000. Gene density in the Giemsa bands of human chromosomes. Chromosome Res. 8, 737–746.

Ferreira, J., Paolella, G., Ramos, C., Lamond, A.I., 1997. Spatial organization of large-scale chromatin domains in the nucleus: a magnified view of single chromosome territories. Cell Biol. 139, 1597–1610.

Filipski, J., Thiery, J.P., Bernardi, G., 1973. An analysis of the bovine genome by Cs_2SO_4-Ag density gradient centrifugation. J. Mol. Biol. 80, 177–197.

Francke, U., 1994. Digitized and differentially shaded human chromosome ideograms for genomic applications. Cytogenet. Cell Genet. 6, 206–219.

Hori, T., Suzuki, Y., Solovei, I., Saitoh, Y., Hutchinson, N., Ikeda, J.E., Macgregor, H., Mizumo, S., 1996. Characterization of DNA sequences constituting the terminal heterochromatin of the chicken Z chromosome. Chromosome Res. 4, 411–426.

Kadi, F., Mouchiroud, D., Sabeur, G., Bernardi, G., 1993. The compositional patterns of the avian genomes and their evolutionary implications. J. Mol. Evol. 37, 544–551.

Lander, E., et al., 2001. Initial sequencing and analysis of the human genome. Nature 409, 860–921.

Macaya, G., Thiery, J.P., Bernardi, G., 1976. An approach to the organization of eukaryotic genomes at a macromolecular level. J. Mol. Biol. 108, 237–254.

Mouchiroud, D., D'Onofrio, G., Aïssani, B., Macaya, G., Gautier, C., Bernardi, G., 1991. The distribution of genes in the human genome. Gene 100, 181–187.

Olofsson, B., Bernardi, G., 1983. Organization of nucleotide sequences in the chicken genome. Eur. J. Biochem. 130, 241–245.

Pavliček, A., Paces, J., Clay, O., Bernardi, G., 2002. A compact view of isochores in the draft human genome sequence. FEBS Lett. 511, 165–169.

Saccone, S., De Sario, A., Della Valle, G., Bernardi, G., 1992. The highest gene concentrations in the human genome are in T-bands of metaphase chromosomes. Proc. Natl. Acad. Sci. USA 89, 4913–4917.

Saccone, S., Cacciò, S., Kusuda, J., Andreozzi, L., Bernardi, G., 1996. Identification of the gene-richest bands in human chromosomes. Gene 174, 85–94.

Saccone, S., Federico, C., Solovei, I., Croquette, M.F., Della Valle, G., Bernardi, G., 1999. Identification of the gene-richest bands in human prometaphase chromosomes. Chromosome Res. 7, 379–386.

Saccone, S., Pavliček, A., Federico, C., Paces, J., Bernardi, G., 2001. Genes, isochores and bands in human chromosomes 21 and 22. Chromosome Res. 9, 533–539.

Sadoni, N., Langer, S., Fauth, C., Bernardi, G., Cremer, T., Turner, B.M., Zink, D., 1999. Nuclear organization of mammalian genomes: polar chromosome territories build up functionally distinct higher order compartments. J. Cell Biol. 146, 1211–1226.

Strouboulis, J., Wolffe, A.P., 1996. Functional compartmentalization of the nucleus. J. Cell Science 109, 1991–2000.

Venter, C., et al., 2001. The sequence of the human genome. Science 291, 1304–1351.

Volpi, E.V., Chevret, E., Jones, T., Vatcheva, R., Williamson, J., Beck, S., Campbell, R.D., Goldsworthy, M., Powis, S.H., Ragoussis, J., Trowsdale, J., Sheer, D., 2000. Large-scale chromatin organization of the major histocompatibility complex and other regions of human chromosome 6 and its response to interferon in interphase nuclei. J. Cell Sci. 113, 1565–1576.

Yokota, H., Singer, M.J., van den Engh, G.J., Trask, B.J., 1997. Regional differences in the compaction of chromatin in human G0/G1 interphase nuclei. Chromosome Res. 5, 157–166.

Zoubak, S., Clay, O., Bernardi, G., 1996. The gene distribution of the human genome. Gene 174, 95–102.