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Web Links

OMIM (Online Mendelian Inheritance in Man). http://www.ncbi.nlm.nih.gov/Omim/

EDN3 (endothelin 3); LocusID: 1908. LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=1908

EDN3 (endothelin 3); MIM number: 131242. OMIM: http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?131242

EDNRB (endothelin receptor type B); LocusID: 1910. LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=1910

EDNRB (endothelin receptor type B); MIM number: 131244. OMIM: http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?131244

GDNF (glial cell derived neurotrophic factor); LocusID: 2668. LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=2668

GDNF (glial cell derived neurotrophic factor); MIM number: 600837. OMIM: http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?600837

RET (ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)); LocusID: 5979. LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5979

RET (ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)); MIM number : 164761. OMIM: http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?164761

SOX10 (SRY (sex determining region Y)-box 10); LocusID: 6663. LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=6663

SOX10 (SRY (sex determining region Y)-box 10); MIM number: 602229. OMIM: http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?602229

GC-rich Isochores: Origin

Intermediate article

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In the interphase nuclei of human and other warm-blooded vertebrates, the most gene-rich chromosomal regions are located in more internal positions and display much more spread-out conformations than the most gene-poor regions, the latter also showing more peripheral nuclear locations.

Article contents

Introduction

In the human genome, gene densities define two 'gene spaces', which have been called the 'empty quarter' and the 'genome core' and represent approximately 85% and 15% of the genome respectively. The genome core is not only characterized by the highest gene density, but also by early replication timing, high recombination frequency and an open chromatin structure, whereas the empty quarter is endowed with opposite properties. (*See* Chromatin Structure and Domains; DNA Replication; Genome Organization of Vertebrates; Isochores.)

The two intragenomic 'major shifts', which occurred separately in the independent ancestral lines of mammals and birds, affected the gene-rich ancestral genome core, which became richer in GC than the empty quarter. The increased thermal stability of deoxyribonucleic acids (DNAs), which characterize the genome core, suggests that a selective advantage was possibly responsible for these changes, mammals and birds being characterized by higher body temperatures than cold-blooded vertebrates. (*See* Evolutionary History of the Human Genome; Genomic and Chromosomal Instability.)

These compositional changes should be considered not only on the basis of the simple DNA sequence, but also on the chromosomal environments where the compositional shifts took place. In fact, the $H3^{+}$ bands, namely the chromosomal bands with the highest concentrations of the most GC-rich H3 isochores, are

endowed not only with a very striking location in the chromosomes (Saccone *et al.*, 1992), but also with a more relaxed chromatin conformation in the interphase nuclei (Saccone *et al.*, 2002). (***See*** Chromosomes 21 and 22: Gene Density; Gene Distribution on Human Chromosomes.)

Compositional Organization of Chromosomes and Nuclei

Some genomic regions of the ancestors of present-day mammals and birds underwent a compositional transition, while others did not (**Figure 1**). The former regions largely correspond to the human $H3^+$ bands, which are prevalently located in the telomeric part of the chromosomes.

Very recent data have shown that the most GC-rich and the most GC-poor DNA of warm-blooded vertebrates, and more precisely DNA characterized by GC levels of more than 50% (H3 isochore family) and less than 38% (L1 isochore family), are located in internal and peripheral regions of the nuclei respectively (Saccone *et al.*, 2002). Thus, DNA probes from H3 and L1 isochores detect not only the two different subsets of chromosomal bands, namely the $H3^+$ and the $L1^+$ bands, but also two different nuclear compartments (**Figure 2**).

The large majority of chromosomes comprise both $L1^+$ and $H3^+$ bands (Federico *et al.*, 2000). It is, therefore, reasonable to assume that most chromosomes contribute to the DNA located in both the internal and the peripheral parts of the nuclei. This is possible because, in the human chromosomes, the most GC-rich $H3^+$ and the most GC-poor $L1^+$ bands are largely located distally and proximally (**Figure 2**), respectively (and are therefore only rarely adjacent), 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 the proposal about a compositional polarity of the interphase chromosomes.

The GC intermediate band DNAs (namely the $L1^-$ and $H3^-$ bands) are characterized by a nuclear

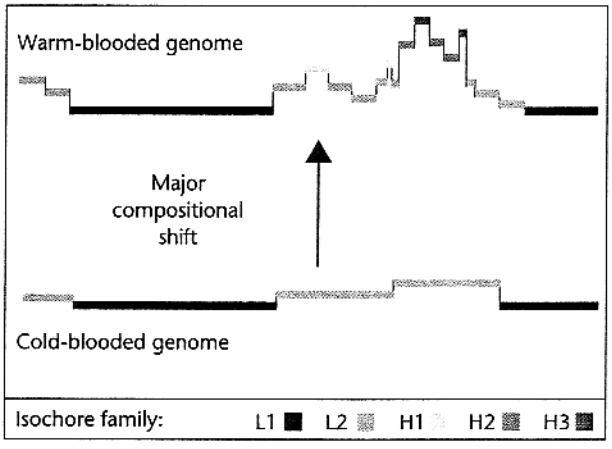

Figure 1 Scheme of GC-rich isochore formation at the deoxyribonucleic acid (DNA) level. During the intragenomic compositional major shifts, the 'genome core' from the ancestors of birds and mammals increased its GC content, allowing the formation of GC-rich isochores, which were shorter and with a much higher compositional fluctuation than the GC-poor isochores of the 'empty quarter'. (Reproduced from Saccone *et al.* (2002).)

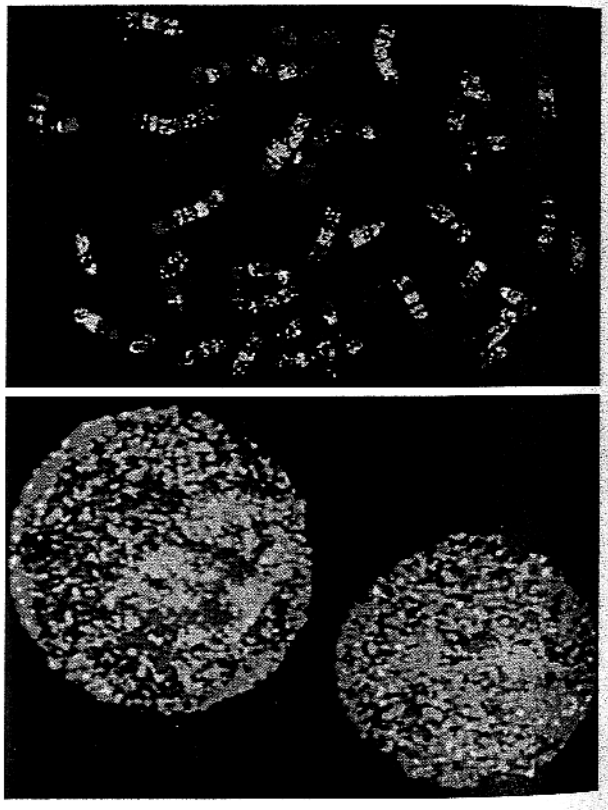

Figure 2 [*Figure is also reproduced in color section.*] Chromosomal and nuclear distribution of the H3 and L1 isochores. Human chromosomes (top) and nuclei (bottom) hybridized with the most GC-rich H3 and the most GC-poor L1 isochores. The H3 and the L1 isochores were labeled with biotin and digoxigenin respectively, and detected with tetramethylrhodamine isothiocyanate (TRITC)–avidin (red signals) and fluorescein isothiocyanate (FITC)–anti-digoxigenin-antibody (green signals) respectively. The yellow color is due to the overlapping of red and green signals. Chromosomes were stained with 4′-6′-diamidino-2-phenylindole (DAPI; blue). The chromosomes show the highest concentrations of H3 and the L1 isochores in telomeric and internal regions respectively (Saccone *et al.*, 1992; Federico *et al.*, 2000). The nuclei show that the DNAs corresponding to the two probes are differentially located, the most GC-rich regions (red signals) being more internal than the most GC-poor ones (green signals).

location similar to those of the $L1^+$ or $H3^+$ bands that are close to them on mitotic chromosomes. This explains why human chromosome 18 shows a peripheral nuclear localization, whereas the human chromosome 19 is located in the nuclear interior (Croft *et al.*, 1999). Indeed, these exceptional situations are due to the fact that these two very small chromosomes lack the most GC-rich $H3^+$ and the most GC-poor $L1^+$ bands respectively.

The Most GC-rich and the Most GC-poor Chromatin Architecture

The two subsets of compositionally opposite bands not only have different locations in the nucleus, but also correspond to different chromatin conformations, the $H3^+$ band DNA being remarkably more relaxed than the $L1^+$ band DNA (**Figure 3**). This is understandable if we consider the special properties of H3 isochores compared with L1 isochores. In fact, the more open chromatin of the most gene-rich $H3^+$ band DNA corresponds to the highest level of transcriptional activity. A number of other data support these findings, the chromatin corresponding to G and R bands of mitotic chromosomes being also more dense and more open, respectively (Croft *et al.*, 1999), and highly expressed sequences extending outside chromosome territories (Volpi *et al.*, 2000). (***See*** Chromatin Structure and Domains; Chromosome Structures: Visualization.)

It should be stressed that the two 'gene spaces', namely 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 the nuclear architecture, which is also demonstrated by other findings. For example, the different replication timing of the DNA located in the internal and

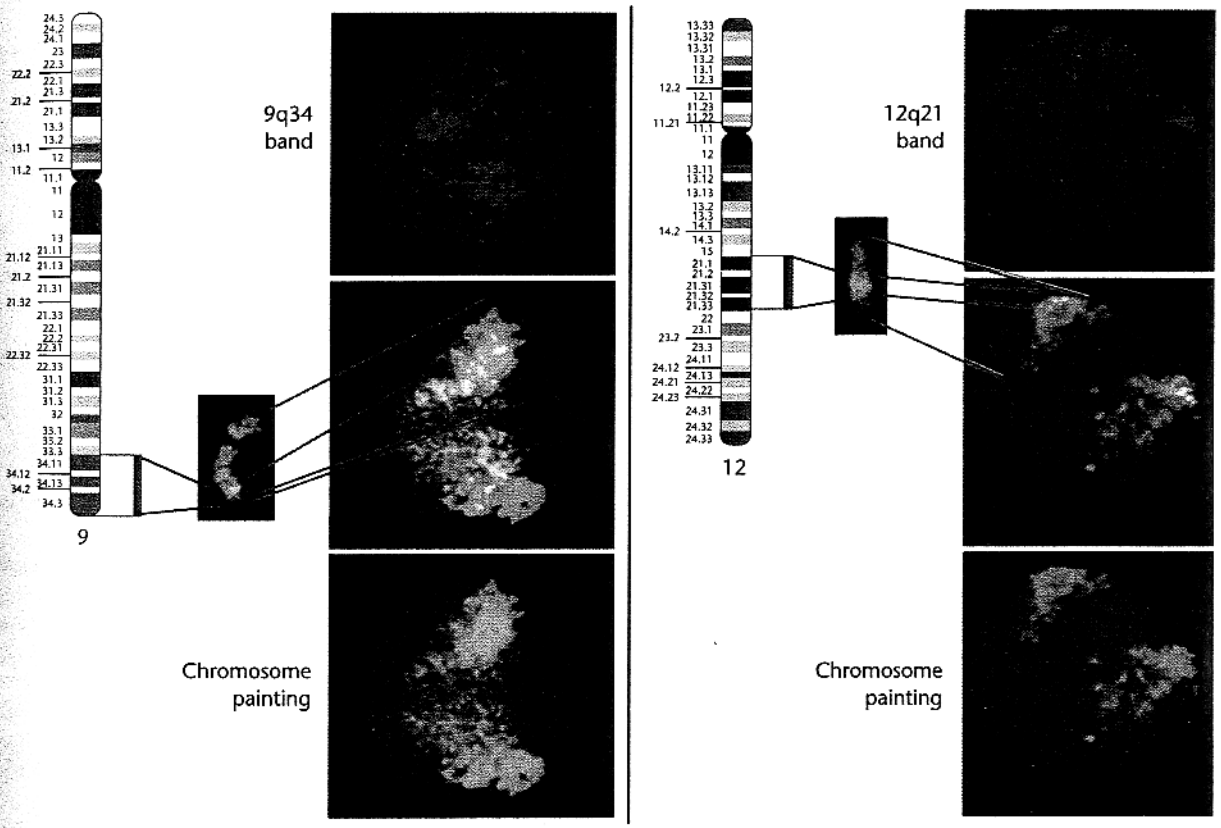

Figure 3 [*Figure is also reproduced in color section.*] Differential compaction of chromatin in the human nuclei. In the nuclei, the most GC-rich band of DNA is very decondensed compared with the most GC-poor band of DNA. This is demonstrated, for example, by *in situ* hybridization, in the interphase nuclei, of the DNA corresponding to the 9q34 (GC-rich) and 12q21 (GC-poor) bands (red signals), together with the relative chromosome painting (green signals). The ideograms of human chromosomes 9 and 12 at 850-band resolution show the $H3^+$ (red) and the $L1^+$ (blue) bands (Saccone *et al.*, 1999; Federico *et al.*, 2000), together with hybridization on the relative mitotic chromosome. There is a different level of compaction, in the nuclei, of the two compositionally opposite bands of DNAs. The upper and lower nuclei show the hybridization of the band DNA (red) and of the chromosome painting (green) respectively. In the middle nuclei, the two images overlap. The nuclei were stained with DAPI (blue). (Modified from Saccone *et al.* (2002).)

peripheral part of the nucleus (Sadoni *et al.*, 1999) is in general agreement with the nuclear location of the most GC-rich $H3^+$ band DNA, which is replicated at the onset of the S-phase, and of the most GC-poor $L1^+$ band DNA, which is replicated at the end (Federico *et al.*, 1998, 2000). (***See*** Chromatin in the Cell Nucleus: Higher-order Organization; DNA Replication.)

Chromatin Organization Leads to Formation of the GC-rich Isochores

The different spatial distribution of genes in the nucleus and the different chromatin compaction, largely related to the levels of transcriptional activity, are general not only for the warm-blooded vertebrates, but possible for all vertebrates, as expected from previous comparative investigations (Bernardi, 2000). The different chromatin compaction of isochores belonging to different families in interphase nuclei suggests a model for the compositional transition from the 'ancestral', modestly 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 the ancestors of 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 already stabilized by the closed chromatin structure (**Figure 4**). This working hypothesis would account for two important features

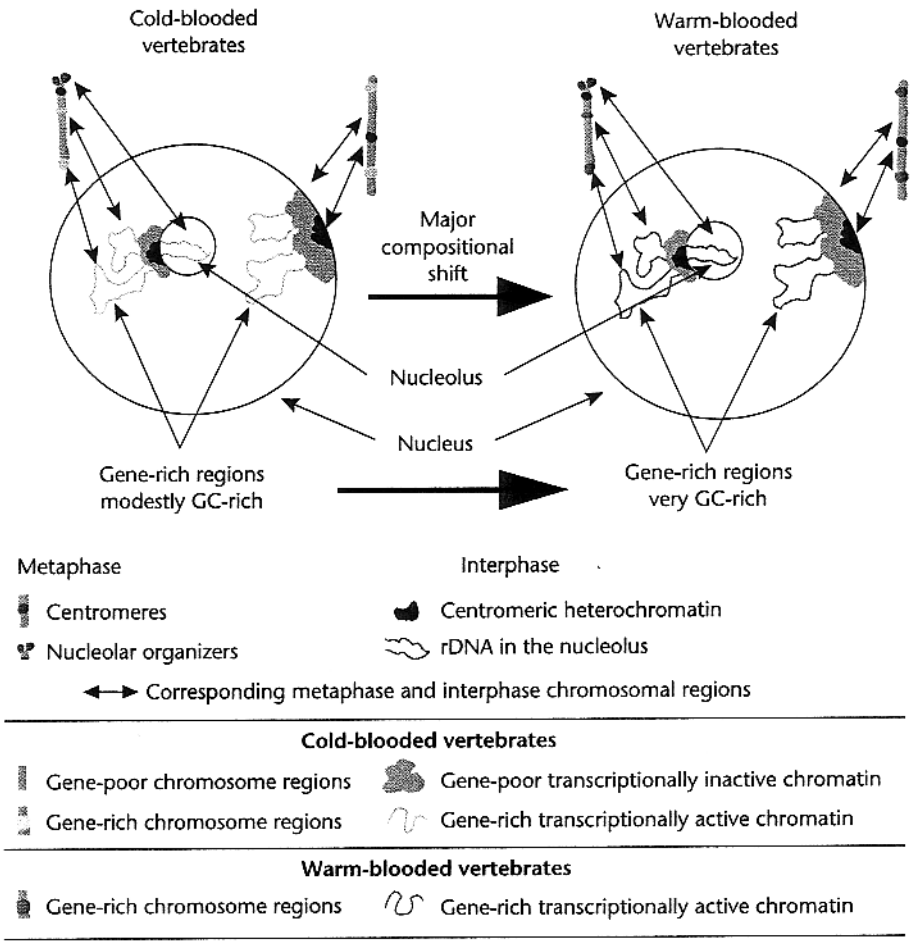

Figure 4 Regional compositional changes in the vertebrate genome. Two chromosomes are represented in their mitotic and interphasic configurations. In warm-blooded vertebrates, the chromosomal regions with the highest concentration of genes are shown (representation of the $H3^+$ bands, the genome core). In the nucleus, these regions are more open relative to the remaining gene-poor regions (empty quarter). If we consider that this could be the situation also in the cold-blooded vertebrates, the GC-rich isochores could arise to enhance their thermal stability, the chromatin being highly decondensed. This stabilization was not needed in cold-blooded vertebrates, because of their lower body temperature, and 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 whole genome. (Modified from Saccone *et al.* (2002).)

of the formation of GC-rich isochores: only the gene-rich minority of the genome, the genome core, underwent the change; and the change affected both coding and noncoding sequences of this part of the genome.

See also
Evolutionary History of the Human Genome
Gene Structure and Organization
Genome Organization

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Gel Electrophoresis

Intermediate article

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Nucleic acids are separated and displayed using various modifications of gel electrophoresis and detection methods. Gel electrophoresis is the core technique for genetic analysis and purification of nucleic acids for further studies. Gel electrophoretic methods provide the highest resolution of all protein separation techniques.

Article contents

Principle of Gel Electrophoresis

Electrophoresis is the migration of charged particles or molecules in an electric field. This occurs when the substances are in aqueous solution. The speed of migration is dependent on the applied electric field strength and the charges of the molecules. Thus, differently charged molecules will form individual zones while they migrate. In order to keep diffusion of the zones to a minimum, electrophoresis is carried out in an anticonvective medium such as a viscous fluid or a gel matrix. Therefore, the speed of migration is also dependent on the size of the molecules. In this way fractionation of a mixture of substances is achieved with high resolution.

Electrophoretic mobility

The electrophoretic mobility is dependent on external factors like electric field strength, viscosity, gel