

Single-Copy Sequence Homology Among the GC-Richest Isochores of the Genomes from Warm-Blooded Vertebrates

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Abstract. We have hybridized a human DNA fraction corresponding to the GC-richest and gene-richest isochore family, H3, on compositional fractions of DNAs from 12 mammalian species and three avian species, representing eight and three orders, respectively. Under conditions in which repetitive sequences are competed out, the H3 isochore probe only or predominantly hybridized on the GC-richest fractions of main-band DNA from all the species investigated. These results indicate that single-copy sequences from the human H3 isochores share homology with sequences located in the compositionally corresponding compartments of the vertebrate genomes tested. These sequences are likely to be essentially formed by conserved coding sequences. The present results add to other lines of evidence indicating that isochore patterns are highly conserved in warm-blooded vertebrate genomes. Moreover, they refine recent reports (Sabeur et al., 1993; Kadi et al., 1993), and correct them in some details and also in demonstrating that the shrew genome does not exhibit the general mammalian pattern, but a special pattern.

Key words: Isochores — Mammals — Birds — Single copy DNA sequences — DNA/DNA hybridization

Introduction

Vertebrate genomes are mosaics of isochores, namely of long, compositionally homogeneous DNA segments that can be subdivided into a small number of families char-

acterized by different GC levels (see Bernardi 1989, 1993a,b, for reviews). In the human genome, which is by far the most extensively studied, the compositional range of isochores is 30–60% GC. Five families of isochores have been identified in this genome: two GC-poor families, L1 and L2, representing together 62% of the genome, and three GC-rich families, H1, H2, and H3, representing 22%, 9%, and 3–5% of the genome, respectively (the remaining DNA is formed by satellite and ribosomal DNAs).

A striking feature of the human genome is that gene concentration is extremely nonuniform and parallels GC levels (Bernardi et al. 1985; Mouchiroud et al. 1991; S. Zoubak and G. Bernardi, paper in preparation). Gene concentration is low and constant over GC-poor isochore families L1 and L2; it increases over GC-rich isochores; and reaches the highest level, more than 20 times that of the GC-poor isochores, in the GC-richest isochore family H3. This family has the highest CpG island concentration, as well as the highest transcriptional and recombinational activities (Aissani and Bernardi 1991a,b; Rynditch et al. 1991), is largely endowed with a distinct, open chromatin structure (see Bernardi 1993b), and is essentially located at T(elomeric) bands (Saccone et al. 1992).

A recent analysis (Sabeur et al. 1993) of the isochore patterns of several species of mammals, belonging to nine out of the 17 eutherian orders, revealed that the isochore organization of the human genome is typical of the “general mammalian pattern.” This is characterized by the presence of sizable amounts (6–10%) of GC-rich isochores, operationally defined as main-band (nonsatellite, nonribosomal) DNA components with a buoyant density higher than 1.710 g/cm^3 , which

essentially correspond to the H3 isochore family and have the highest density of CpG islands (Aïssani and Bernardi 1991a,b). This general pattern is shared by eight out of nine orders investigated, with only *Pholidota* (an order comprising a single genus whose representative is pangolin) showing a different isochore pattern (Sabeur et al. 1993). Two other special patterns were, however, recognized in *Megachiroptera* and *Myomorpha*, two suborders belonging to the orders *Chiroptera* and *Rodentia*, respectively. Other suborders of these orders exhibit the general pattern. Along the same line of research, the recent analysis of the isochore pattern of species belonging to eight avian orders has demonstrated the presence of a single isochore pattern, which is also characterized by the presence of GC-rich isochores (Kadi et al 1993).

When GC levels of third codon positions of pairs of mammalian homologous genes sharing either the general pattern, or the myomorph pattern, are plotted against each other, very high correlation coefficients are found, with regression lines having slopes that are very close to unity (Bernardi et al. 1988; Mouchiroud and Bernardi 1993). The extremely high compositional similarity of third codon positions indicates (Bernardi et al. 1985; Aïssani et al. 1991) a compositional similarity of the isochores, in which the corresponding genes are located. Similar, but more limited, observations were made on avian genes (Kadi et al. 1993). In contrast, the same plots for homologous genes from genomes exhibiting the general and the myomorph pattern, respectively, showed slopes different from unity. In particular, the genes located in H3 isochores in the general pattern are characterized by higher GC value in third codon positions and in the isochores hosting those genes. Correlation coefficients remained, however, very high (Bernardi et al. 1988; Mouchiroud et al. 1988, 1991; Mouchiroud and Bernardi 1993), indicating that, in spite of compositional differences, the compositional ranking order of third codon positions of homologous genes is largely preserved. Finally, when third codon positions from homologous genes from man and chicken were compared, a surprisingly high degree of compositional similarity was found (Kadi et al. 1993; see also Bernardi 1993b).

Obviously, the very rigorous approach based on the compositional properties of third codon positions can only be applied to species for which a large enough number of homologous sequences are available. For the majority of mammals and birds, it is only possible, however, to rely on investigations at the DNA level (Sabeur et al. 1993; Kadi et al. 1993). The most interesting, GC-richest and gene-richest isochores of the mammalian and avian genomes, are, however, often found in DNA fractions that also contain satellite DNAs that are usually, but not necessarily, GC-rich. This complicates the assessment of the amount of main-band DNA in those fractions, which is the main criterion

used for assigning a given genome a certain compositional pattern.

A way of circumventing this problem has been developed here by investigating whether the single-copy sequences of the H3 isochores (which do not cross-hybridize with satellite DNAs nor with ribosomal DNA) share homology with the single-copy sequences of isochores characterized by comparable GC levels from other warm-blooded species that belong to different orders. This approach has allowed us to refine the reports of Sabeur et al. (1993) and Kadi et al. (1993), and also to correct them in some details and in demonstrating that the shrew genome does not share the general mammalian pattern but shows another special pattern.

Materials and Methods

The compositional fractions of mammalian and avian DNAs used in this work were those already described (Sabeur et al. 1993; Kadi et al. 1994), except for three series of fractions from human, mouse, and calf DNAs, respectively, that were specially prepared for this work, using a DNA load per tube of 10 A_{260} units, and a molar BAMD/nucleotide ratio (r_j) of 0.14 (BAMD is bis(acetato-mercuri-methyl)dioxane).

The probes used were: (i) fraction 8 from the fractionation of human DNA presented in Fig. 1A; this fraction is a good representation of the human H3 isochore family; (ii) a human ribosomal DNA (rDNA) probe (a gift of Dr. J. Kusuda, National Institute of Health, Tokyo, Japan); (iii) the human Alu repeat present in pBlur8 (Jelinek et al. 1980). Probes were labeled by random-priming, using α^{32} -dCTP, to a specific activity of 10^8 cpm/ μ g. Each DNA fraction (0.5 μ g) was transferred to a Bio-Rad membrane using a slot blot apparatus (Bio-Rad) according to the manufacturer's instructions. In the case of human DNA fractions, loads were only 50 ng. Hybridizations were performed according to a standard procedure (Sambrook et al. 1989). Filters were washed for 20 min at room temperature in $2\times$ standard saline citrate (SSC), 0.1% sodium dodecyl sulphate (SDS), and then for 20 min in $0.1\times$ SSC, 0.1% SDS at 68°C. Hybridizations with the human H3 probe were performed using as competitors a $100\times$ excess of rDNA, a $100\times$ excess of human Cot 1 DNA (Gibco-BRL), and a $100\times$ excess of human DNA depleted of H3. Cot 1 DNA is a sonicated DNA fraction obtained by denaturation and reassociation to an (initial DNA concentration \times time) value equal to 1. Human DNA depleted of H3 was obtained by pooling fractions 1–7 from the fractionation experiment of Fig. 1A. Preannealing of repetitive sequences was performed by incubating the denatured probe with competitor DNAs at 37°C for 20 min. Exposure times for autoradiography were 48–72 h at -72°C .

Results

Single-Copy Sequence Hybridization of Human H3 Isochores on Compositional DNA Fractions of Warm-Blooded Vertebrates

Table 1 presents a list of the 12 mammalian species (representing eight out of the 17 eutherian orders) and of the three avian species (representing three orders) that were investigated here by hybridization with a human H3 probe (fraction 8 of Fig. 1A). Figure 1A–C displays the

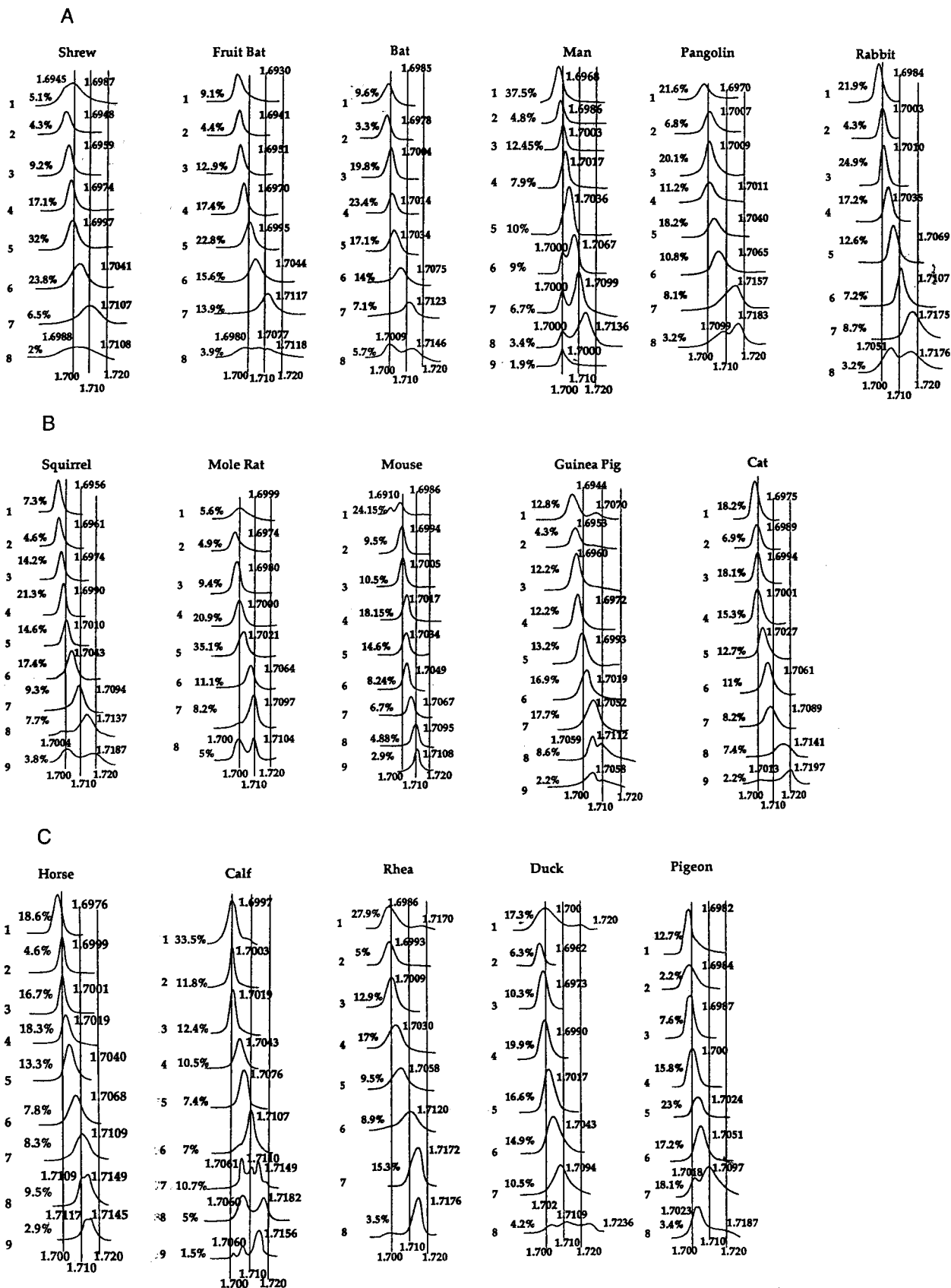


Fig. 1. A-C Analytical CsCl profiles of fractions from preparative Cs₂SO₄/BAMD density gradient of mammalian and avian DNA fractions. The relative amounts of DNA and the modal buoyant densities of the peaks are indicated.

Table 1. Classification of mammalian^a and avian species^b investigated in present work

Order sub- and infra-order	Family	Species investigated	
Mammalian			
Insectivora	Soricidae	<i>Crocidura</i> sp.	Shrew
Chiroptera			
Megachiroptera	Pteropidae	<i>Pteropus</i> sp.	Fruit bat
Microchiroptera	Vespertilionidae	<i>Myotis myotis</i>	Bat
Primates	Hominidae	<i>Homo sapiens</i>	Man
Pholidota	Manidae	<i>Manis</i> sp.	Pangolin
Lagomorpha	Leporidae	<i>Oryctolagus cuniculus</i>	Rabbit
Rodentia			
Sciurognathi			
Sciuromorpha	Sciuridae	<i>Sciurus vulgaris</i>	Squirrel
Myomorpha	Spalacidae	<i>Spalax</i> sp.	Mole rat
	Muridae	<i>Mus musculus</i>	Mouse
Histricognathi			
Caviomorpha	Caviidae	<i>Cavia porcellus</i>	Guinea pig
Carnivora	Felidae	<i>Felis domesticus</i>	Cat
Perissodactyla	Equidae	<i>Equus caballus</i>	Horse
Artiodactyla	Bovidae	<i>Bos taurus</i>	Calf
Avian			
Rheiformes	Rheidae	<i>Rhea americana</i>	Rhea
Anseriformes	Anatidae	<i>Cairina moschata</i>	Duck
Columbiformes	Columbidae	<i>Columba livia</i>	Pigeon

^a From Nowak and Paradiso (1983). Rodents are classified according to Webb et al. (1977)

^b From Perrins and Middleton (1985)

analytical CsCl density profiles of mammalian and avian DNA fractions from preparative Cs₂SO₄/BAMD density gradients (Sabeur et al. 1993; Kadi et al. 1993; and present work).

The hybridization signals obtained by using human fraction 8 from Fig. 1A as a probe on fractionated mammalian DNAs were strongest in the GC-richest fractions of all the genomes studied, but signals were also present in the GC-poor genome compartments (e.g., see Fig. 2). These results could be expected because DNA from human fraction 8 includes both single-copy and repetitive sequences (such as SINEs, LINEs, and rDNA) that may contribute to the observed hybridization patterns.

Indeed, rDNA contributes to the signals detected after hybridization with fraction 8, as it does cross-hybridize to the GC-richest fractions of all genomes (data not shown). This result could be expected from the base composition, the location in Cs₂SO₄/BAMD fractions (Zerial et al. 1986; Aissani and Bernardi 1991a) and the known interspecific sequence homology of rDNA. A 100× excess of cold human rDNA was found, however, to suppress effectively all signals due to rDNA (data not shown).

In contrast, cross-hybridization with Alu sequences, that are known to be more concentrated in the human GC-rich isochores (Soriano et al. 1983), including the H3 family (Zerial et al. 1986), than in the rest of the genome, were only detected in a few cases and after a long exposure time (data not shown). This class of repetitive sequences, which is very abundant in the human genome, is, therefore, divergent enough in prima-

ry structure not to contribute significantly to the hybridization patterns exhibited by DNA fractions from the other species.

Interestingly, fraction 9 of human DNA, which only contains the 1.700 g/cm³ satellite, was positive in the hybridization experiment, as expected on the basis of the fact that fraction 8 also contained part of this satellite.

In any case, the signals derived from the repetitive sequences present in the human fraction 8 (which include both SINEs and LINEs as well as the 1.700 g/cm³ satellite DNA) were suppressed by a competition step using a combination of human Cot 1 DNA (100× excess) and human DNA depleted of H3 (fractions 1–7 of Fig. 1A; 100× excess). The single-copy sequence hybridization patterns so produced showed that the signals from the GC-poor fractions are effectively suppressed, while those from the GC-richest fractions are still present and are concentrated in one-two fractions (Fig. 2). Expectedly, in the case of human DNA, all signals were suppressed, except those localized in fraction 8 (Fig. 2). In the other cases (bat and shrew), the last fractions did not show any hybridization because they are largely formed by satellite and by rDNAs. The hybridization conditions of Fig. 2 were, therefore, used in all other experiments (Figs. 3–5).

In assessing the buoyant densities of the GC-richest main-band components, several factors were taken into account: (i) the relative intensity of the hybridization signals of DNA fractions and the relative DNA amounts present in the fractions; since DNA loads on filters were the same for all fractions, signals were "enriched"

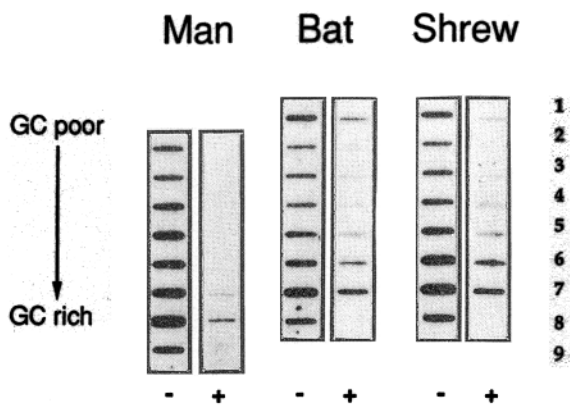


Fig. 2. Slot blot hybridization patterns obtained using as a probe fraction 8 from human DNA (see Fig. 1A) in the absence [lanes marked with a minus (-) symbol] or in the presence [lanes marked with a plus (+) symbol] of repeated-sequence competitor DNA (see Materials and Methods for hybridization conditions). Species names are indicated at the top of each lane. The modal buoyant densities of the fractions increase from the top to the bottom, as indicated at the left of the figure, the last fractions often containing, however, satellite DNAs characterized by low buoyant densities. Fraction numbers are indicated at the right of the figure. Fraction 1, the pellet, of human DNA was not investigated.

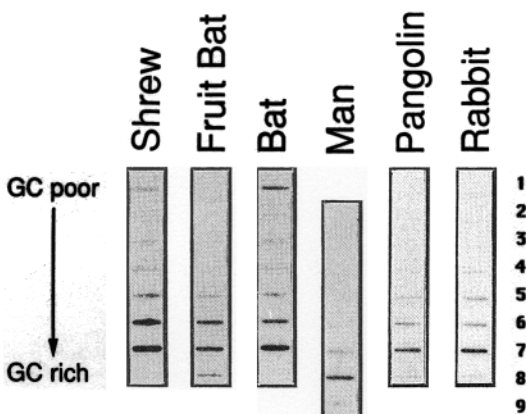


Fig. 3. Slot blot hybridization patterns obtained using as a probe fraction 8 of human DNA (see Fig. 1A) on fractionated mammalian genomes. Other indications as for Fig. 2.

in the case of fractions containing small amounts of DNA compared to those containing large amounts; (ii) the presence of satellite DNAs, which did not hybridize the probe but reduced the actual amount of main-band DNA present in a fraction; and (iii) the asymmetries of some peaks. These factors were dealt with as described in the following two sections.

Localization of Human H3 Isochores on Fractionated Mammalian DNAs

Figures 3 and 4 display these experiments which concern DNA fractions from Fig. 1A-C. In the case of *shrew*, hybridization of fraction 8 from human DNA (see Fig. 1A) was strongest in fraction 6 and, more so, in fraction 7. At first sight, this would indicate that the

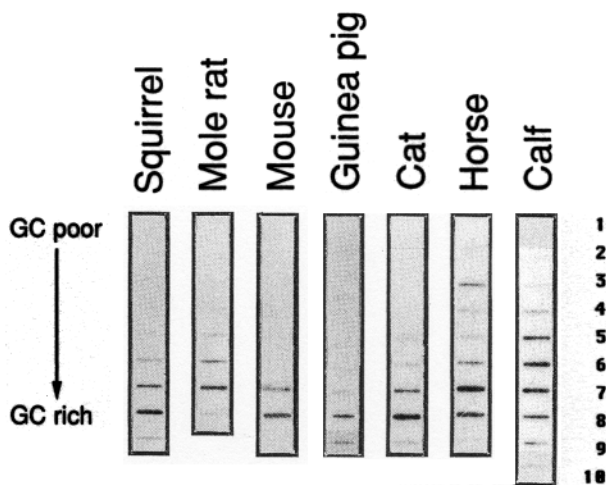


Fig. 4. Slot blot hybridization patterns obtained using as a probe fraction 8 of human DNA (see Fig. 1A) on fractionated mammalian genomes. Other indications as for Fig. 2.

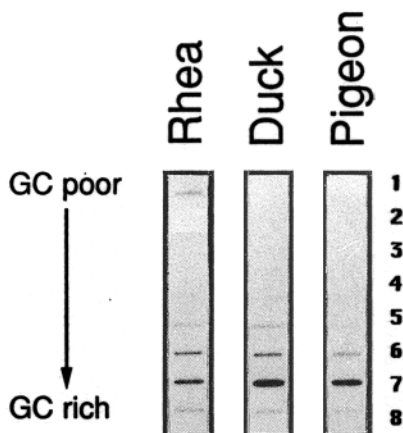


Fig. 5. Slot blot hybridization patterns obtained using as a probe fraction 8 of human DNA (see Fig. 1A) on fractionated avian genomes. Other indications as for Fig. 2.

peak of the hybridizing shrew DNA is in fraction 7, a fraction having a modal buoyant density of 1.7107 g/cm^3 , thought to correspond to the H3 isochores by Sabeur et al. (1993). There is, however, a large difference in the relative DNA amounts present in these two hybridization-positive fractions. While fraction 7 only represents 6.5%, fraction 6 represents 23.8% of shrew DNA (Sabeur et al. 1993; incidentally, the latter value was erroneously indicated as 3.8% in the original paper; likewise, fraction 5 was 32.0% and not 3.2%, and fraction 4 was 17.1% and not 7.1%). The DNA loads on the filter being the same for all fractions, the signal of fraction 7 was, therefore, "enriched" almost fourfold relative to that of fraction 6, a factor larger than the difference in hybridization intensity. In other words, the shrew DNA sequences cross-hybridizing with human H3 are not only shared by fractions 6 and 7, but are, in fact, more abundant in fraction 6. The modal buoyant density of these sequences are, therefore, certainly higher than 1.7044 g/cm^3 (the modal buoyant density of

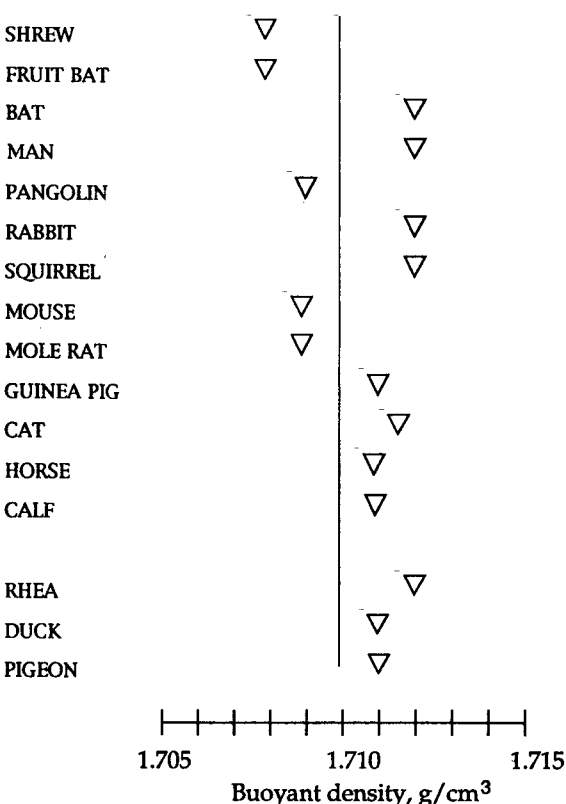


Fig. 6. Schematic representation of the hybridization patterns observed. Arrows indicate best estimates of buoyant densities of main-band DNAs hybridizing single-copy sequences from human H3 isochores. In the case of rhea DNA, there is some uncertainty about the buoyant density assignment (see text). In the case of duck and pigeon genomes, hybridizing fractions trail toward higher buoyant densities (not indicated in figure).

fraction 6), but lower than 1.7107 g/cm^3 (the buoyant density of fraction 7, a fraction characterized by a very broad CsCl profile trailing on the heavy side and likely to contain some cryptic satellite DNA), probably in the neighborhood of 1.708 g/cm^3 . As already mentioned, fraction 8 did not show any hybridization signal indicating that this very heterogeneous fraction is essentially formed by satellite DNAs and by rDNA (Sabeur et al. 1993).

The fruit bat genome showed the highest hybridization in fraction 6 and, more so, in fraction 7. This fraction was estimated to be mainly made of a satellite DNA (1.7117 g/cm^3), but was suspected to hide main band DNA, which should have a buoyant density intermediate between those of fractions 6 (1.7044 g/cm^3) and 7 (1.7117 g/cm^3), possibly 1.708 g/cm^3 . This is the buoyant density of the small central peak appearing in fraction 8 (Sabeur et al. 1993), which is very weakly positive as far as hybridization is concerned.

The bat genome showed the strongest signal in fraction 7 which corresponds to a main band component centered at 1.7123 g/cm^3 . Some hybridization positive material was, however, also present in fraction 6 (1.7075 g/cm^3) which contains about twice the amount of DNA

of fraction 7, indicating a signal "enrichment" in the latter fraction. Incidentally, the signal present in the pellet is, in all likelihood, derived from a contamination from the GC-richest fractions, which are the last ones to be collected through the needle whose tip is positioned just above the pellet.

Pangolin DNA showed the strongest hybridization in fraction 7, (1.7157 g/cm^3) which is mainly formed by a satellite DNA (1.7183 g/cm^3), also present in fraction 8, which is hybridization negative. Fraction 7 shows a 1.709 g/cm^3 main-band DNA shoulder, and this component is also present in fractions 6 and 8. This 1.709 g/cm^3 component appears to be responsible for the hybridization.

In the case of rabbit, the strongest hybridization was in fraction 7, which is mainly formed by a satellite DNA characterized by a very high buoyant density (1.7175 g/cm^3), also present, together with another satellite, in fraction 8, which is practically negative in the hybridization experiment. The main-band DNA of fraction 7 should be higher than 1.7107 g/cm^3 , which is the modal buoyant density of fraction 6, a fraction that shows an extremely low signal.

Squirrel DNA showed the strongest hybridization in fraction 8, which exhibits a modal buoyant density of 1.7137 g/cm^3 . This fraction is, however, skewed on the GC-rich side, suggesting that the buoyant density of main-band component corresponding to human H3 is lower, possibly around 1.712 g/cm^3 . Fraction 7 was less strongly positive, as expected from its CsCl profile which extends up to 1.714 g/cm^3 , whereas fraction 9, mainly formed by satellite and possibly rDNA, was practically negative, also as expected.

The strongest hybridization signals of mouse DNA were found in fraction 7 and, more so, in fraction 8, that have modal buoyant densities of 1.7067 and 1.7095 g/cm^3 , respectively.

The case of mole rat was very similar to that of mouse, the strongest signal being concentrated in fraction 7, a fraction containing main band DNA centered at 1.7097 g/cm^3 , and, to a lesser extent, in fraction 6 (1.7064 g/cm^3).

Guinea pig DNA showed the highest hybridization in fraction 8 which contained, next to a satellite DNA (1.7059 g/cm^3), a 1.7112 g/cm^3 main-band DNA component (Sabeur et al. 1993).

The cat genome showed the strongest signal in fraction 8, whose modal buoyant density is 1.713 – 1.714 g/cm^3 . The profile of fraction 8 is, however, very broad and its modal buoyant density is much higher than that of the preceding fraction 7 (1.7089 g/cm^3), in all likelihood due to the presence of a satellite DNA which is the predominant component of fraction 9 and has a buoyant density of 1.7197 g/cm^3 . Under these circumstances, the buoyant density of the cat DNA component hybridizing to human H3 is likely to be in the neighborhood of 1.711 – 1.712 g/cm^3 . A weaker signal was

found in fraction 7, whose CsCl profile was centered at 1.7089 g/cm^3 , but trailed toward higher values.

Horse DNA showed the strongest signals in fraction 7 and, less so, in fraction 8. The former is made up of a main-band component centered at 1.7109 g/cm^3 , the latter of a satellite DNA (1.7149 g/cm^3) and of a 1.7109 g/cm^3 component, which was mistakenly considered a satellite DNA by Sabeur et al. (1993). Fraction 9, comprising two satellite DNAs (1.7117 g/cm^3 and 1.7145 g/cm^3), did not show any hybridization.

The calf genome showed the strongest hybridization signals in fraction 5, and, more so, in fractions 6 and 7, that were centered at 1.7107 and 1.711 g/cm^3 , respectively. The best estimate for the buoyant density of the main-band component of calf DNA which correspond to human H3 is, therefore, 1.711 g/cm^3 .

Localization of Human H3 Isochores on Fractionated Avian DNAs

These experiments, shown in Fig. 5, concern DNA fractions from Fig. 1C. Rhea DNA showed hybridization with human H3 DNA on fractions 6 and 7, that show modal buoyant densities of 1.7120 and 1.7172 g/cm^3 , respectively, the highest intensity being in fraction 7 (Fig. 5). Fraction 7 is mainly formed by a satellite DNA (1.7172 g/cm^3), which also forms the majority of the hybridization-negative fraction 8, and exhibits a CsCl profile skewed on the light side. This suggests that the modal buoyant density of the main-band DNA present in fractions 6 and 7 is around 1.712 g/cm^3 .

Duck DNA showed hybridization with human H3 DNA mainly on fraction 7 (Fig. 5), which had a modal buoyant density of 1.7094 g/cm^3 but trailed toward higher values (Kadi et al. 1993). This component may correspond to the central peak (1.7109 g/cm^3) of fraction 8.

Pigeon DNA showed hybridization with human H3 DNA on fraction 7 (Fig. 5) which had a bimodal profile due to the presence of both a light satellite (1.7018 g/cm^3) and main band DNA (1.7097 g/cm^3), which trailed toward the GC-rich side (Kadi et al. 1993) and might be assigned a buoyant density of 1.711 g/cm^3 .

Figure 6 presents a scheme of the estimated buoyant densities of main-band DNA components from the genomes of warm-blooded vertebrates investigated here which hybridize single-copy sequences from human fraction 8.

Discussion

The results obtained in the present work largely confirm the conclusions reached by Sabeur et al. (1993) and by Kadi et al. (1993) about the buoyant densities of the GC-richest isochores of the genomes of warm-blooded vertebrates. They, however, refine those results and also

correct, to some extent, the conclusions. Moreover, they provide information on single-copy sequence homology in warm-blooded vertebrates.

The Mammalian Isochore Patterns

The existence of a general mammalian isochore pattern (Sabeur et al. 1993) is confirmed on the basis of the single copy sequence homology among the GC-richest isochores from mammalian genomes. Indeed, the single-copy sequences present in the human H3 isochore probe hybridized on DNA components having buoyant densities in the expected 1.711 – 1.712 g/cm^3 range for bat, rabbit, squirrel, guinea pig, cat, horse, and calf—seven species representing six orders.

The only case in which the general pattern was not confirmed was that of shrew. The different conclusion reached here is based on the fact that the majority of hybridizing DNA from shrew is not in fraction 7 (where a 1.7017 g/cm^3 component was considered to correspond to the very GC-rich isochores of the general pattern), but in fraction 6. Sabeur et al. (1993) already pointed out, however, that the DNAs from the insectivores that they investigated (shrew, mole, and hedgehog) showed very different modal buoyant densities, that of shrew showing the lowest value of all mammalian DNAs except for that of fruit bat. In the present work, the shrew genome was definitely characterized as not belonging to the general pattern, but representing another special pattern, which adds to those of *Pholidota*, *Megachiroptera*, and *Myomorpha*. Like that of shrew, all these other special patterns, which were confirmed by the present work, are characterized by the fact that their GC-richest isochores reach a lower GC level, corresponding to buoyant densities of 1.708 – 1.709 g/cm^3 , than the GC-richest isochores of the genomes belonging to the general pattern, which shows buoyant densities of 1.711 – 1.712 g/cm^3 . The existence of no less than four special patterns (plus a general pattern) in mammals is in sharp contrast with the single pattern observed in birds (see below).

Although the highest buoyant density levels reached are similar, the special patterns are not identical with each other, as already indicated by Sabeur et al. (1993). The myomorph pattern (exhibited here by the mouse and the mole rat genomes) is characterized by a very narrow compositional distribution and by a higher modal buoyant density of total DNA compared to the general pattern (Sabeur et al. 1993). In contrast, the patterns of the shrew genome and, more so, of the fruit bat genome, show an overall shift toward lower modal buoyant densities. Finally, the pangolin pattern more closely resembles the myomorph pattern than the other two special patterns.

While the pangolin genome cannot be compared with other genomes from the same order, because pangolin belongs to the only genus present in the order *Pholido-*

ta, this comparison can be done in the other cases. This shows that species belonging to other suborders of the insectivores, chiropters, and rodents exhibit the general mammalian pattern (in contrast to shrew, fruit bat, and myomorpha, respectively). This suggests two major alternatives: (i) either the paleontological viewpoint (see Carroll 1988; Novacek 1992) concerning the monophylies of insectivores, chiropters, and rodents is correct, and then one should suppose that the compositional patterns of the mammalian orders under consideration have undergone changes in some suborders, but not in other ones and that the compositional transitions leading to the special patterns are relatively recent ones; or (ii), the special patterns are as ancient or more ancient than the general pattern, and the paleontological viewpoint is incorrect as is the traditional taxonomy. This dilemma (which was already discussed by Sabeur et al. 1993) cannot be solved at present.

A few additional corrections of the previous results (Sabeur et al. 1993) concern (i) the nonsatellite nature of the 1.7109 g/cm³ component of fraction 8 from horse DNA; (ii) the fact that the H3 equivalent of rabbit DNA is located in fraction 7 and not 6; and (iii) the absence of DNA components equivalent to H3 in the last fraction of shrew, bat, cat, and horse, in which small amounts of main-band DNA were supposed to exist.

The Avian Isochore Patterns

As far as the results of hybridization of human H3 DNA on avian DNAs are concerned, the agreement of the present results with the duck and pigeon data of Kadi et al. (1993) is quite satisfactory. In contrast, the present data indicate, however, that the highest concentration of main-band rhea DNA hybridizing to human H3 is in fraction 7, hidden by the very abundant 1.717 g/cm³ satellite, and not in fraction 6. The present conclusion is that the modal buoyant density of the rhea DNA component corresponding to human H3 is close to 1.712 g/cm³ (see Results), a value coinciding with the previous estimate (Kadi et al. 1993); however, this conclusion needs to be confirmed because of the vast amounts of satellite DNA present in fraction 7. Obviously, this may make uncertain the similarity of the isochore pattern of rhea with those of other birds. The results obtained with ostrich DNA (Kadi et al. 1993) support, however, the similarity of isochore patterns of both paleognathous and neognathous birds.

It should be stressed that in all avian genomes studied, the amount of DNA centered at about 1.711–1.712 g/cm³ is more abundant than in the mammalian genomes (Kadi et al. 1993) and that there is a definite trend for these GC-richest components to trail toward higher buoyant densities. These facts are in agreement with the finding that third codon positions of coding sequences from the chicken genome attain higher GC values than those of man (Kadi et al. 1993).

General Implications

If one recalls that the DNA loads on the filters were 10 times lower for human fractions than for those from the other species, it is clear that the present results are in agreement with the expected sequence divergences among the genome of warm-blooded vertebrates. Still, the hybridization signals obtained indicate a conservation of single-copy sequences during the evolution of the species investigated. This conservation is likely to concern essentially, if not exclusively, coding sequences. Indeed, the average substitution frequencies of third codon positions for genes from man and calf, for example, are about 20% (Bernardi et al. 1993), that of second codon positions about 4%, and that of first codon position around 10%. This indicates an average sequence identity of only about 65%. It is known, however, that (i) different genes exhibit large fluctuations about the average value, covering a wide range in the case of third codon positions (Bernardi et al. 1993) and that (ii) synonymous and nonsynonymous substitutions are correlated for the same genes (paper in preparation). Under these circumstances, it is reasonable to think that the sequences that are essentially responsible for the present results are conserved coding sequences characterized by matches well above the average value of 65%. This suggestion is supported by the abundance of CpG islands in the GC-richest DNA fractions of warm-blooded vertebrates (Aïssani and Bernardi 1991a,b), their preferential association with housekeeping genes (Gardiner-Garden and Frommer, 1987) and the generally conserved nature of housekeeping genes. Some contribution of other, noncoding, single-copy sequences having strong similarity with the human counterparts cannot be ruled out. However, such sequences are known to show a stronger divergence compared to coding sequences. Therefore, their contribution cannot be an important one.

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