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SOME PROPERTIES OF THE MAJOR COMPONENTS OF THE MOUSE GENOME.

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The technology of in vitro recombinant DNA has already achieved a major breakthrough in the field of the organization of the eukaryotic genome by demonstrating that, contrary to all expectations, eukaryotic genes may contain nucleotide sequences which are not represented in the mRNA transcripts which are translated into proteins. It is quite clear, however, that this new approach may also provide other original insights into the more general problem of genome organization in eukaryotes.

This problem has been mainly studied, so far, by investigating the kinetics of reassociation of DNA. Such an approach has shown that segments of eukaryotic genomes can be classified into different families according to their rate of reassociation; furthermore, it has shown some interspersion relationships among DNA segments belonging to different families.

Another experimental approach used in this area has consisted in resolving native DNA fragments by centrifugation in density gradients. Such a method has proven very useful for the separation of "satellite" DNAs and of repetitive genes (see, for instance, refs. 1-7). More important, density gradient centrifugation has revealed that the bulk of mammalian DNA is formed by three major components, which can be separated from each other (2-4). These components comprise : 1) a main component forming 50 to 65 % of total mammalian DNA ; in different mammals, this ranges in buoyant density from 1.697 to 1.701 g/cm^3 and can be resolved, in several species, into two sub-components ; 2) a component forming 20-25 % of total DNA, and having a buoyant density of 1.704 g/cm^3 ; 3) a component forming about 10 % of total DNA and having a buoyant density of 1.708 g/cm^3. Major components similar to those found in mammalian DNAs appear to be present in avian DNAs, whereas reptilian, amphibian and fish DNAs exhibit a decreasing asymmetry of their CsCl bands ; invertebrate DNAs show symmetrical CsCl bands. The findings just outlined indicate the existence of phylogenetic differences at the macromolecular level in the organization of eukaryotic genomes, and raise the problem of the evolutionary origin of the major components of mammalian DNAs.

In the present work, we have investigated the organization of nucleotide sequences in the major DNA components of mouse and the localization of certain genes in such components. The former study has been done by using two different experimental approaches aiming at two different levels of organization : 1) we have investigated

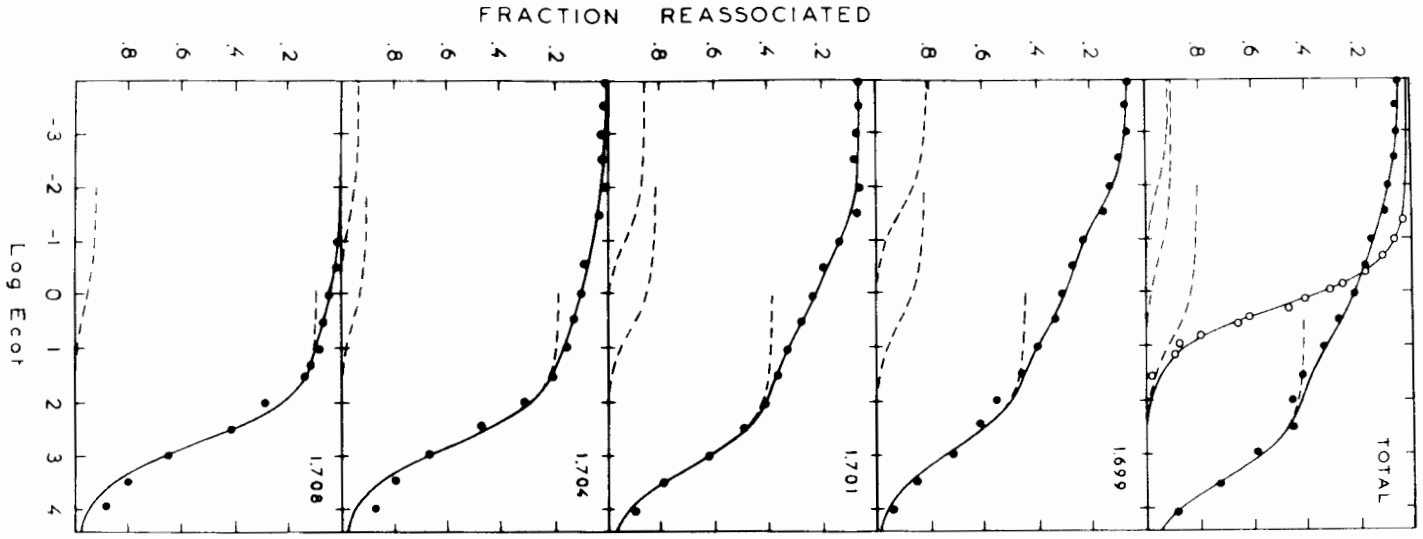

Fig. 1 Reassociation kinetics of total DNA and major DNA components from mouse (●), and E.coli DNA (○). DNA samples were degraded to fragments of about 300 base pairs by Hae III and Hpa II (●) or by Hae III alone (○), and were reassociated at T_m-25° in 0.2 M potassium phosphate, (KP), 10^{-4}M EDTA, for most Ecot values above 1, or in 5 mM KP, 10^{-4}M EDTA, and various KCl concentrations for lower Ecot values. Ecot values are cot (the product of initial DNA concentration, c_o, by reassociation time, t) values adjusted to 0.18 M K^+. For each point, about 2 ug of DNA are loaded on a small, (0.1 ml), hydroxyapatite column, and single stranded and double stranded DNA are eluted by a linear KP gradient (0.03 M to 0.4 M, at 50°). The extent of reassociation is measured in a Zeiss spectrophotometer by monitoring the column effluent at 260 nm. The solid line through the points is calculated by a computer program as the best fit for ideal second order kinetics ; dashed lines represent kinetic classes.

the reassociation kinetics of each DNA component, making use of the ability of hydroxyapatite columns to discriminate single-stranded from double-stranded DNA fragments (8) ; 2) we have analyzed the frequency of short nucleotide sequences in each DNA component by studying the frequency of 5'terminal dinucleotides released by spleen acid DNAase (9). As far as the localization of certain genes is concerned, we will report here one example, that of the ß-globin gene.

The reassociation kinetic results (fig. 1) indicate a striking difference in the relative amount of "single-copy" and repetitive nucleotide sequences in the major components of mouse DNA. Intermediate-repetitive sequences are almost absent from the 1.704 and 1.708 g/cm^3 components, whereas they are concentrated in the two sub-components of the main, light component (1.699 and 1.701 g/cm^3). This finding suggests that the two types of interspersion of intermediate repetitive and unique sequences, (short period and long period interspersion)(10), are located on different DNA components.

The frequency of 5'terminal dinucleotides released by spleen acid DNase from each DNA component varies, as expected, with their base composition. The difference histograms obtained by subtracting in each case the frequency of 5'terminal dinucleotides released by degrading bacterial DNAs having the same base composition as the mouse DNA components show, however, the same patterns for all components (fig. 2). These data have an important implication, if one considers the reassociation results of fig. 1, and recalls that "satellite" DNAs have terminal dinucleotide patterns which are very largely different from those of the major components (fig. 2 and paper in preparation). In fact, the very similar 5'-terminal dinucleotide patterns found in components having very different levels of intermediate repetitive sequences do not support the suggestion that such sequences derive from "satellite" DNA sequences (11), and rather favor their origin from single-copy sequences.

Finally, the ß-globin gene appears to be localized on the 1.701 g/cm^3 component. In fact, the ß-globin cDNA excised from plasmid MßI (fig. 3) hybridizes on Hind III fragments having molecular weights of $5.9.10^6$ and $0.75.10^6$ (fig. 4), in fair agreement with recent results from another laboratory (12).A very weak hybridization on fragments of identical molecular weight present on the 1.699 g/cm^3 component may be due to cross contamination from the 1.701 g/cm^3 component. Interestingly, a faint hybridization (not visible in fig. 4) was detected in the 1.708 g/cm^3 component on a fragment having a molecular weight of $1.4.10^6$. It will be interesting to see whether this corresponds to a ß-minor gene, (13), of relatively recent evolutionary origin.

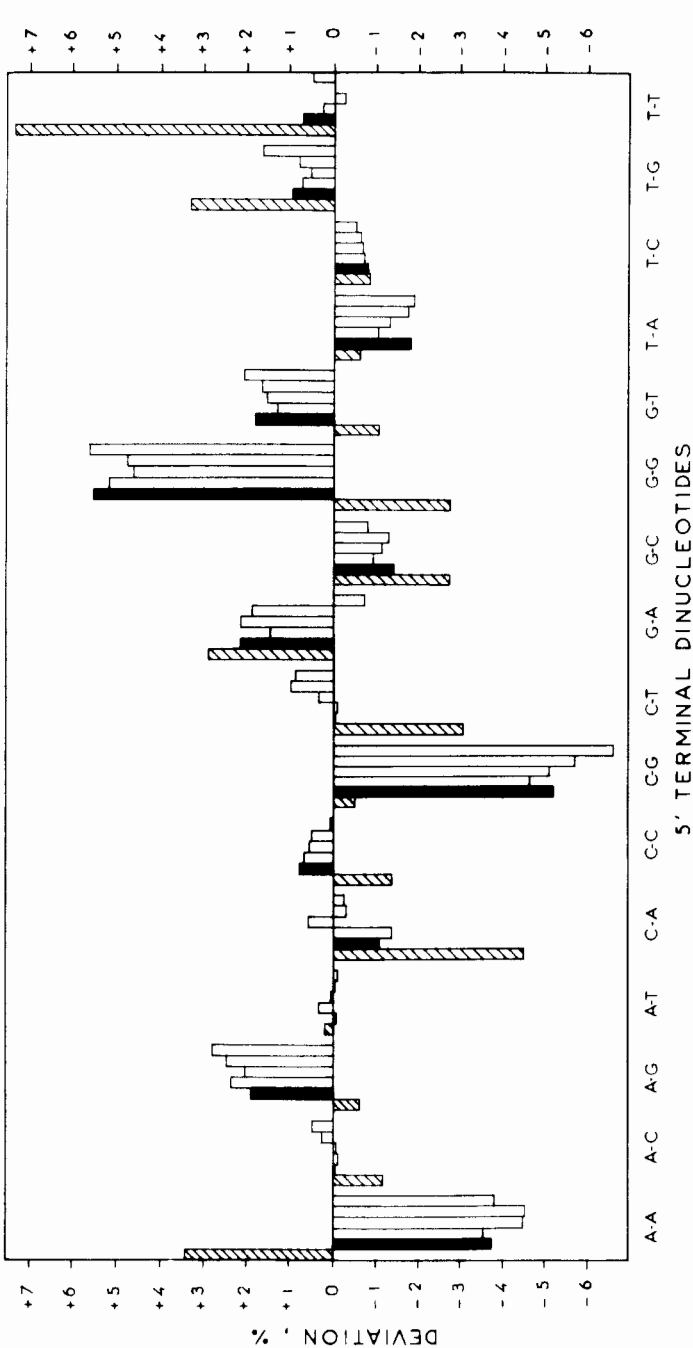

Fig. 2 Difference histograms of 5'-terminal dinucleotides of total DNA, (black bars), satellite DNA, (cross-hatched bars), and major DNA components (white bars ; left to right : 1.699, 1.701, 1.704, 1.708) from mouse. The histograms represent the differences between the percentages of each 5'-terminal dinucleotide as found in mouse DNA and in bacterial DNAs having the same base composition. 5'-terminal dinucleotides were determined on spleen acid DNase digests, showing 10 % and 20 % hyperchromic shift, according to Bernardi and Gaillard (14).

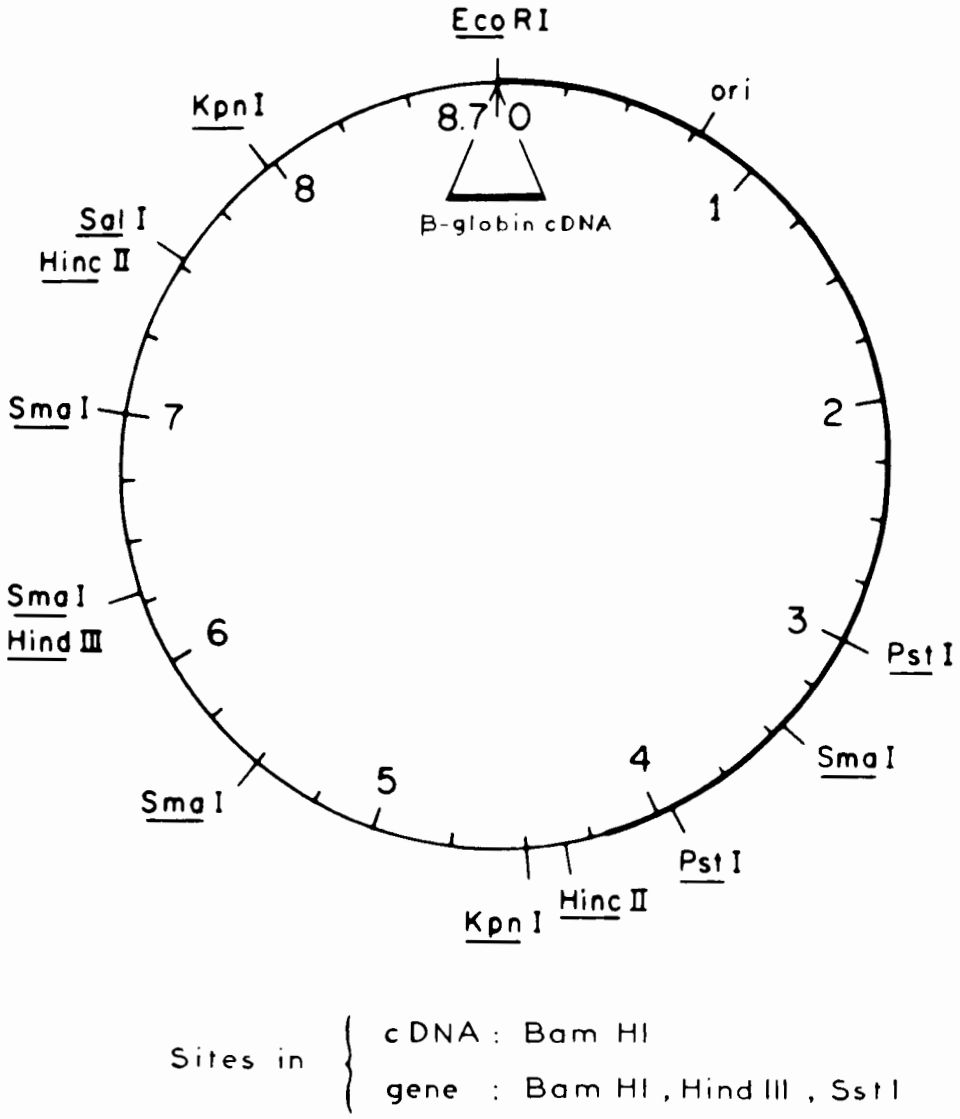

Fig. 3 Physical map of the Mβ1 plasmid. The cDNA insert (about 530 base pairs) is localized at the Eco RI site of plasmid pcRI. The insert contains a Bam HI site (15) ; the gene contains, in addition, a Hind III and Sst I site (12) in the internal nucleotide sequence not represented in the mRNA. Figures inside the circle indicate molecular weights in millions. The pcRI map is taken from ref. 16.

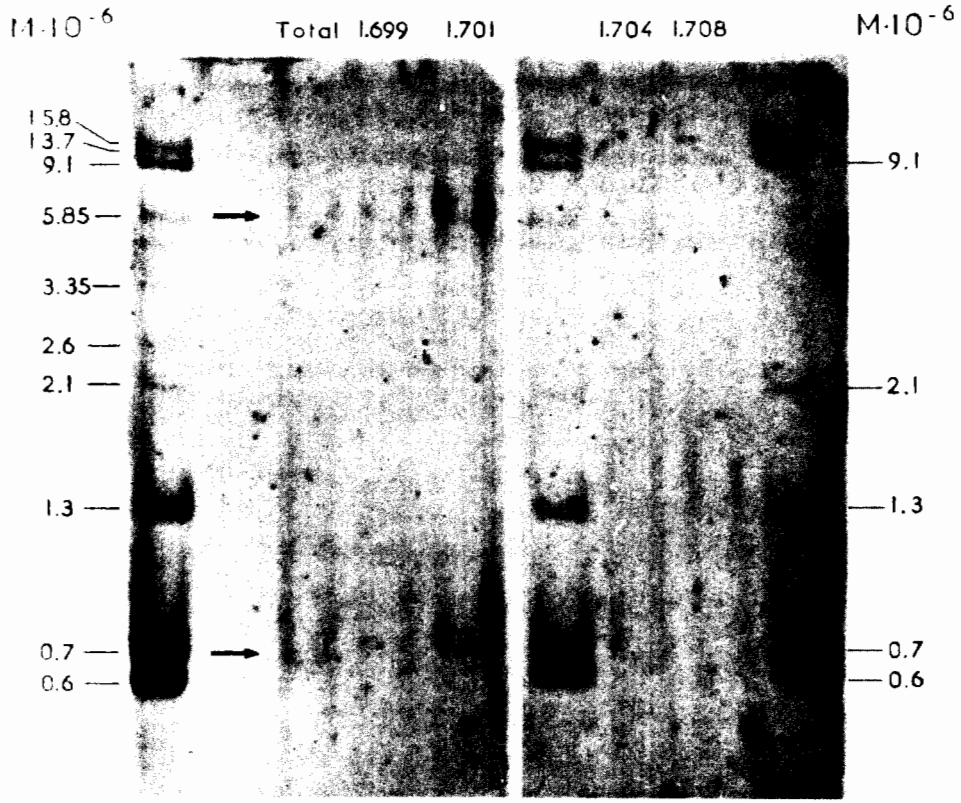

Fig. 4 Detection of β-globin genes in total DNA and major DNA components from mouse. 30 µg of total DNA, 30 µg of 1.699, 42 µg of 1.701, 21.6 µg of 1.704, 9.6 µg of 1.708, (the amounts of DNA components are 4 times larger than their amounts in total DNA), were digested with Hind III, fractionated by electrophoresis on 1 % agarose slab gels, denatured, blotted on nitrocellulose sheets, and hybridized, (17), with the Hha I fragment of Mβ1 plasmid, containing cDNA from mouse β-globin messenger RNA, and labelled by nick-translation with ^{32}P to a specific activity of about 2.10^8 c.p.m. / µg. Marker DNAs were cDNA-containing fragments obtained by degrading Mβ1 with Bam HI, ($9.1.10^6$), Kpn I ($5.85.10^6$), Bam HI+ Hind III + Pst I ($3.6.10^6$ and $2.6.10^6$), Hha I ($1.3.10^6$), Bam HI + Hha I ($0.7.10^6$ and $0.6.10^6$).

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