Gene distribution and nucleotide sequence organization in the mouse genome

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Mouse DNA was fractionated by preparative centrifugation in density gradients of Cs₂SO₄ containing 3,6-bis(acetamidomerciurethyl)dioxane (BAMD). The effects of temperature, BAMD/nucleotide molar ratio and solvent on the fractionation were explored. The fractions so obtained were investigated by analytical centrifugation in CsCl density gradient and by hybridization with a number of gene probes. These approaches led to the definition of satisfactory conditions for the rapid fractionation of mouse DNA; (b) to the localization of a number of genes in mouse DNA fractions; and (c) to a better understanding of the mosaic organization of the mouse genome and, more specifically, to a better estimate of both the intermolecular and intramolecular compositional heterogeneity of mouse DNA in the (75 – 150) x 10⁵-base size range.

Equilibrium density gradient centrifugation of DNA in the presence of ligands, like Ag⁺ or BAMD [3, 6-bis(acetamidomerciurethyl)dioxane], is a powerful method for the resolution of DNA molecules which differ in base composition and/or nucleotide sequence [1 – 11]. This experimental approach was shown to be very effective not only for isolating satellite DNAs [1 – 6] but also for fractionating main-band DNA from mammalian genomes [2]. This observation was the starting point for a series of detailed investigations [3, 4, 7 – 9] which have led to the discovery of a compositional compartmentalization in the genomes of warm-blooded vertebrates. Indeed, neglecting satellite and minor components, like ribosomal DNA, the nuclear DNA from warm-blooded vertebrates can be resolved, over a wide molecular size range (3 – 300) x 10⁶ bases (3 – 300 kb; [4]), into a small number of major components. These correspond to maxima of DNA distribution in the gradient [4], and are characterized by intermolecular heterogeneities comparable with those of bacterial DNAs having the same molecular mass and the same G + C content [8].

A Gaussian analysis of CsCl profiles of DNA from fractions obtained by preparative density gradient centrifugation in the presence of Ag⁺ or BAMD has led to the identification of four major components comprising: (a) two light components, characterized by modal buoyant densities in the 1.698 – 1.702 g/cm³ range (all buoyant densities quoted in this article were determined by analytical centrifugation in CsCl density gradients), and representing about 2/3 of main-band DNA, these components cannot be separated in some genomes [3]; and (b) two heavy components having modal buoyant densities equal to 1.704 g/cm³ and 1.708 g/cm³, respectively, and representing together the remaining 1/3 of main-band DNA. The heavy components account for the strong heterogeneity and marked asymmetry of main-band DNAs from warm-blooded vertebrates [2 – 4, 7 – 9]. In contrast with the picture just presented, main-band DNAs from the vast majority of cold-blooded vertebrates show weak heterogeneities, only slightly skewed CsCl peaks and major components which are only or mainly in the same buoyant density range as the light components of warm-blooded vertebrates [3, 10, 11].

The reassociation kinetics [12] and the localization of specific families of interspersed repeats [13 – 15] have shown that major components are characterized by distinct sequence organizations, namely by different amounts of interspersed repeats and by different interspersion patterns. These differences concern both major components from the same genome and isoplastic major components from different genomes.

The families of molecules forming the major components are derived, by the mechanical and enzymatic breakage which unavoidably accompanies DNA preparation, from much longer DNA segments, which have been called isochores [8]. These have an average size well above 200 kb [4, 7], are fairly homogenous in base composition [4, 8, 12] and seem to correspond to the DNA segments present in Giemsa and Reverse chromosomal bands [8]. In the human genome these DNA segments have been estimated to have an average size of 1250 kb [16].

Isolated major components were used to study the genome distribution of several genes, proviral and repetitive sequences [7, 13 – 15, 17 – 23]. This approach has led to new insights on the genome of warm-blooded vertebrates [23]. Very briefly, the base composition and ratio of CpG to GpC in both coding and non-coding sequences, as well as codon usage, have been shown to be highly non-uniform and the genome of warm-blooded vertebrates.
Since the isolation of major components is both time-consuming and expensive, we decided to fractionate the mouse DNA by using a single-step Cs$_2$SO$_4$/BAMD density gradient centrifugation in order to locate a number of specific sequences directly in the fractions so obtained. The study of several experimental conditions allowed us to optimize the fractionation of mouse DNA in a given range of G+C content. The results presented in this work shed new light on the mosaic organization of the mouse genome. More specifically they allow a better estimate of both (a) intramolecular heterogeneity of mouse DNA in the 75–150-kb size range and (b) the intermolecular heterogeneity of mouse DNA major components. The accompanying paper [24] reports a similar investigation on the human genome.

**MATERIALS AND METHODS**

**DNA preparations**

Two DNA preparations (1 and 2) were obtained from the liver of female 6-week-old Balb-c mice essentially by using the detergent method [8]. The average size of DNA molecules in these preparations was 75 kb as determined by sedimentation, electrophoretic mobility and band width in CsCl density gradient. DNA from phage λ was obtained from the bacterial culture service of our Institute.

**Preparative centrifugation of DNA in Cs$_2$SO$_4$/BAMD density gradient**

5.152 g Cs$_2$SO$_4$ (Merck, Darmstadt, FRG) were added to either 0.1 M Na$_2$SO$_4$, 5 mM Na$_2$B$_4$O$_7$, pH 9.4, or 0.4 M Na$_2$SO$_4$, 20 mM Na$_2$B$_4$O$_7$, pH 9.4. The two solvents will be referred to as the 0.1 M and the 0.4 M solvent, respectively, and contained 10 A$_{260}$ units DNA, unless otherwise stated. A 1 mM solution of BAMD was then added to obtain the desired [BAMD]/[DNA P] molar ratio, r$_f$. The final density was 1.47 g/cm$^3$ in the 0.1 M solvent and 1.50 g/cm$^3$ in the 0.4 M solvent. DNA solutions had a volume of 9 ml and were overlaid with paraffin oil in capped polycarbonate tubes. Centrifugations were carried out at three different temperatures (8°C, 20°C, 30°C) in a Beckman L5-50B preparative ultracentrifuge, using a 50 Ti rotor at 36000 rpm for 68 h. After centrifugation, tubes were emptied with a stainless-steel needle, slowly immersed in the solution using a rack-and-trinoid system; care was taken not to touch or stir the DNA-BAMD complex pelleted at the bottom of the tube. The solution was pumped out with a peristaltic pump (flow rate, 10 ml/h) and its absorbance was monitored at 253.7 nm with a Uvicord (LKB, Stockholm, Sweden). Fractions of 0.2 ml were collected and assembled, and their absorbance at 260 nm was measured. The pellet being first dissolved in 3 ml of 10 mM Tris, pH 7.4, 1 mM EDTA.

The fractions and redissolved pellet were dialyzed against 10 mM Tris pH 7.4, 10 mM EDTA at 20°C once and then dialyzed against 10 mM Tris pH 7.4, 1 mM EDTA at 4°C three times in order to release BAMD from DNA.

As in previous work [5–7], the BAMD used here was a gift of Dr H. Bünnemann [25].

**Preparative CsCl density gradient centrifugation**

Solid CsCl (Suprapur; Merck, Darmstadt, FRG) was added to DNA from Cs$_2$SO$_4$/BAMD fractions, dissolved in 10 mM Tris pH 7.4, 1 mM EDTA to obtain a final density of 1.700 g/cm$^3$. 9-ml solutions were loaded into polycyliner tubes and overlaid with paraffin oil. Centrifugation and fraction collection were performed as described above.

**Analytical CsCl density gradient centrifugation**

Fractions from the preparative gradients (after several rounds of dialysis; see above) containing 0.03 A$_{260}$ unit DNA in 0.4 ml 10 mM Tris pH 7.4, 1 mM EDTA were supplemented with solid CsCl to obtain a density of 1.700 g/cm$^3$. Analytical centrifugations were made using a Spinco model E analytical ultracentrifuge equipped with a monochromator, mirror optics, an electronic scanner and a multiplexer; a modification of the multiplexer by G. Macaya and M. Grosjean permitted the simultaneous study of nine DNA samples, using double-sector cells and a six-hole titanium rotor. Phase 2C, q = 1.742 g/cm$^3$ [26] was used as a density marker and a value of 1.19 x 10$^6$ cm$^2$ g$^{-1}$ s$^{-1}$ was taken for the buoyant gradient constant $b_p$ (see [5]). The modal buoyant densities of DNA bands could usually be defined within $\pm 0.0002$ g/cm$^3$.

The intermolecular (or buoyant density) heterogeneity was estimated by using the equation given by Schmid and Hearst [27]: $\Delta \delta = \Delta \delta_B + \Delta \delta_A$.

**Restriction enzyme digestion and hybridization**

DNA samples (10 µg) from the fractions of Cs$_2$SO$_4$/BAMD preparative gradients were digested with restriction endonucleases (Boehringer, Mannheim, FRG) using the conditions given by the manufacturer. Electrophoresis was carried out on 0.8% agarose (Bio-Rad Laboratories, Richmond, CA) horizontal gels, using 8.9 mM Tris, 8.9 mM H$_2$BO$_3$ and 2.5 mM EDTA, pH 8.3, as the solvent. DNA was then denatured and transferred to nitrocellulose filters (BA85, DSS, Dassel, FRG) as described [28]. Filters were prehybridized in plastic bags for 3 h at 65°C in 6 x standard saline citrate (SSC), 0.5% sodium dodecyl sulfate (SDS), 5 x Denhardt's solution (Denhardt's solution contains 0.02% each of polyvinylpyrrolidone, bovine serum albumin fraction V, Sigma, St Louis, MO, and Ficol, Pharmacia, Uppsala, Sweden; use of denatured salmon sperm DNA was avoided because of cross-hybridization with the actin probes. Hybridization was carried out at 65°C for 20 h in the prehybridization solution containing the 32P-radiolabeled probe (100 ng, specific radioactivity = 5.0 x 10$^8$ – 1.0 x 10$^9$ cpm/µg DNA), labeling was done by nick translation [29]. Filters were first washed in 2 x SSC, 0.5% SDS at 65°C, and finally rinsed in 1 x SSC, 0.1% SDS at 65°C (low stringency), or in 0.1 x SSC, 0.5% SDS at 65°C (high stringency). Filters were then dried and exposed to X-ray film in the presence of intensifying screens at −70°C for 1–7 days.

**Probes**

The w-abl probe, obtained from Dr C. Croce, was a 1.6-kb BglII fragment corresponding to the 2.5–4.1-kb segment on the map of the Abelson leukemia virus [30]; this fragment had been inserted into pBR322 plasmid, using EcoRl linkers.

The mouse skeletal, cytoplasmic and cardiac actin probes, obtained from Dr D. Buckingham, were cDNAs (pAM91, pAL41 and pAF81 respectively) inserted into the PstI site of pBR322 plasmid [31–33].

The mouse α and β-globin probes, obtained from Dr. C. Weissmann, were cDNAs inserted into the EcoRI site of pGRII plasmid [34].
Fig. 1. Fractionation of mouse DNA (preparation 1) by Cs₂SO₄/BAMD density gradient centrifugation. DNA was centrifuged at the temperature and $r_f$ values indicated, using the 0.1 M solvent. The top panel shows the transmission profiles of fractionated DNA as recorded at 253.7 nm. Corresponding fractions from nine ($r_f = 0.14$) or six tubes ($r_f = 0.16$ and $r_f = 0.19$) were pooled. The middle panel shows the histograms of the relative amounts of DNA in the fractions, fraction 1 corresponds to the pellet, the hatched box indicating satellite DNA. The bottom panel shows the analytical CsCl profiles of total, unfraccionated DNA and of pooled fractions. See also Materials and Methods and the Results.

RESULTS

Fractionation of mouse DNA by Cs₂SO₄/BAMD density gradient centrifugation

The effects of three parameters, temperature, $r_f$ and solvent, on the resolution of mouse DNA were explored in three series of experiments.

In the first series of experiments (Fig. 1) the Cs₂SO₄/BAMD preparative centrifugation was run at 20°C. The results so obtained provided the following information.

- The amount of pelleted DNA increased from 24% to 50% and to 57%, respectively, on increasing the $r_f$ values from 0.14 to 0.16 and to 0.19. Satellite DNA, $q = 1.691$ g/cm³, which represents 7% of mouse DNA [8], was predominantly (at $r_f = 0.14$), or exclusively (at $r_f = 0.16$, or $r_f = 0.19$) present in the pellet. At $r_f = 0.19$ the satellite was not found in the redissolved pellet, either because the formation of an irreversible satellite-DNA-BAMD complex at high $r_f$ had increased the density of the satellite and moved it into the main band or because of the insolubility of the satellite-DNA-
BAMD complex as obtained at high \( r_t \). The first explanation is more likely to be correct in view of the other cases of irreversible binding and increased density of satellite DNA (1.692–1.694 g/cm\(^3\) instead of 1.691 g/cm\(^3\)) shown in Figs 2 and 3 (see also below).

b) Increasing the \( r_t \) value decreased the amount of DNA in the density gradient from 76% to 50% and to 43%, respectively, and led to a better resolution of (G + C)-rich fractions. As expected, the CsCl buoyant density of the first fraction from the gradient increased with increasing \( r_t \), whereas the most (G + C)-rich fractions had in all cases a buoyant density of 1.710–1.711 g/cm\(^3\), with only minute amounts of DNA having higher G + C levels.

c) The last fractions in the gradients showed (a) the presence of some DNA banding at about 1.703 g/cm\(^3\), this might be identical with the minor 1.704 g/cm\(^3\) component detected in previous work [3]; (b) the presence of a very broad main peak, this is apparently related to both a larger compositional heterogeneity, probably due to contamination of the last fractions by the preceding ones, and a lower molecular mass, due to the enrichment of low-molecular-mass molecules in the tails of the DNA band from the preparative gradient; (c) a lower amount of DNA compared to what was expected from the absorbance measurements, this seems to be due to an overestimate of the low-concentration DNA present in these fractions.

In the second series of experiments (Fig. 2) the Cs\(_2\)SO\(_4\)/BAMD preparative centrifugation was run at 30°C in the 0.4 M solvent. This solvent provided a better resolution of the DNA molecules having the highest BAMD load (which otherwise would have been pelleted), because it shifted the center of distribution of DNA towards the gradient range exhibiting a lower slope compared with the 0.1 M solvent. A comparison of the results obtained at different \( r_t \) values among themselves and with the previous ones showed the following.

a) The amount of pelleted DNA increased, with increasing \( r_t \), from 3% to 11% and to 37%, respectively. In the first case, \( r_t = 0.12 \), very little DNA was pelleted; the pellet was remarkably heterogeneous and contained only part of the satellite DNA, which was otherwise distributed over many fractions for reasons which were not studied further. In the other two cases, pellets comprised the totality of the satellite, but contained much smaller amounts of DNA (11% and 37%) compared with those found at 20°C at the same \( r_t \) values, (24% and 50% respectively). It should be noted that satellite DNA, as redissolved from the pellets obtained at \( r_t = 0.14 \) and \( r_t = 0.16 \), showed higher buoyant densities than expected; the same phenomenon was also found in the experiments of Figs 1 and 3; this suggests that under certain experimental conditions BAMD binding may be partially irreversible.

b) Because of the smaller amounts of pelleted DNA, the DNA concentration in the gradient was much higher; this was accompanied by a lower resolution of (G + C)-rich DNA and by a higher resolution of (A + T)-rich DNA compared with the experiments done at 20°C. As a consequence, the highest buoyant density attained was lower, 1.7082 g/cm\(^3\); as expected, this material showed a CsCl peak which was asymmetric on the heavy side and corresponded to about 10% of DNA, a much larger amount than that found at 20°C. The absorbance profile obtained with the preparative Cs\(_2\)SO\(_4\)/BAMD centrifugation at 20°C (Fig. 1) was characterized by a main peak, corresponding to a CsCl buoyant density of 1.702–1.704 g/cm\(^3\), flanked by a left and a right shoulder. The left shoulder corresponded to DNA ranging in buoyant density from 1.700 g/cm\(^3\) to 1.703 g/cm\(^3\), the right one comprised DNA centered at 1.708–1.710 g/cm\(^3\). While this roughly trimodal distribution could still be seen in the experiment run at 30°C (Fig. 2), under these conditions the left shoulder had the shape of a peak, owing to the fact that less (A + T)-rich DNA was pelleted at 30°C; in contrast, the right shoulder was changed into a very sharp peak, and comprised about 10% of material centered at about 1.708 g/cm\(^3\).

A third series of experiments was aimed at understanding the phenomena just described. Since this striking change in absorbance profiles seemed to be related to the higher temperature used in the experiment of Fig. 2, another experiment was run at 8°C, \( r_t = 0.16 \), in the 0.4 M solvent (Fig. 3).
Under these conditions 30% of the DNA was pelleted, a result comparable with those which could be obtained at 30°C, $r_t = 0.15 - 0.16$, or at 20°C, $r_t = 0.14 - 0.15$. The profile was, however, much more symmetrical, but a right shoulder corresponding to 10% of DNA, banding at 1.708 - 1.710 g/cm³, was present.

A control experiment (Fig. 4) was done by centrifuging an intact homogeneous DNA from phage $\lambda$ at 30°C and 8°C, $r_t = 0.14$. In this case a sharp additional peak appeared at 30°C, but was absent at 8°C. In the case of mouse DNA, by contrast, changing the temperature from 30°C to 8°C only altered the shape of the peak, which took the appearance of a shoulder (compare Figs 2 and 3). Interestingly, in spite of the change in shape of the absorbance profiles, the 10% of mouse DNA present at the top of the gradient showed the same modal buoyant density at 30°C and 8°C, except for some small extent of contamination indicated by the lower buoyant densities of the last dilute fractions from the second-order experiments (a) and (b). These results suggest that, in the centrifugation of mouse DNA at 30°C, the sharp peak corresponds to the superposition of an artefact similar to that shown by $\lambda$ DNA and of a real fractionation.

An experiment was performed to investigate the buoyant density of the DNA contained in the sharp peak representing the heaviest 10% of the mouse DNA. The most (G+C)-rich fractions (8–10) from the experiment of Fig. 2b were pooled and recentrifuged at 20°C, $r_t = 0.14$ in a Cs₂SO₄/BAMD density gradient using 0.1 M solvent. This recentrifugation allowed a further resolution for the high-density fractions and also established that the highest buoyant density value attained, for 1% of mouse DNA, was 1.7116 g/cm³ (Fig. 5).

The hybridization pattern of single-copy genes in mouse fractions

Fractions from the experiment of Fig. 1a were used to locate the c-abl oncogene, the skeletal and cardiac $\alpha$-actin genes and the $\alpha$- and $\beta$-globins, using high-stringency conditions. The $\nu$-abl probe showed (Fig. 6a) a hybridization band on a fragment of 21.5 kb from fraction 7 (1.7074 g/cm³) and a
very faint hybridization on the following fraction 8 (1.7096 g/cm³). The cardiac α-actin probe showed (Fig. 6b) a main hybridization band on a fragment of 4.3 kb from fraction 4 (1.7022 g/cm³); very faint bands could be also detected in the neighboring fractions 3 (1.7008 g/cm³) and 5 (1.7041 g/cm³). The skeletal α-actin probe hybridized on a fragment of 8.2 kb from fraction 7 (Fig. 6c) and showed a very faint hybridization on fraction 8 (1.7096 g/cm³); in other words, the hybridization behavior of the skeletal α-actin probe was identical to that of the ν-actin probe. Finally the β-globin probe hybridized on a fragment from fraction 4 and the α-globin probe on a fragment present in fractions 7 and 8 (1.7096 g/cm³) (data not shown). In every case the fragments had the sizes expected from the literature.

A striking feature of all these experiments was the very narrow distribution of hybridization across the gradient, most of the radioactivity being essentially found in a single fraction; neighboring fractions, at most 2 mg/cm³ apart in modal buoyant density, showed very little or no hybridization with the probes.

The hybridization pattern of the actin multigene family in mouse DNA fractions

The actin probes were also used, at low stringency, to detect the genes and pseudogenes of the actin family in fractions from the preparative Cs₂SO₄/BAMD gradients. The result obtained with the skeletal α-actin probe on the fractions from the centrifugation carried out at 30°C, rₑ = 0.16 (Fig. 2c), is shown in Fig. 7. Data from this experiment, as well as those obtained with hybridization of the skeletal α-actin probe with fractions from centrifugation carried out at 20°C, rₑ = 0.14 (Fig. 1a), and the cytoplasmic β-actin probe with fractions from centrifugation carried out at the former conditions (Fig. 2c), are presented in Table 1. Fig. 7 shows that the skeletal α-actin probe hybridizes on over 40 fragments localized in all the fractions from the preparative centrifugations. These fragments are known to be largely scattered over all mouse chromosomes [21].

These results deserve four main comments.

a) Experimental conditions were different from those of Fig. 5 to allow the detection of weaker sequence homologies; this led to photographic saturation for some bands.

b) The resolution of hybridizing fragments on the fractions was much better than that obtained with unfractonated...
DNA, as expected. In the latter case the hybridization bands correspond to the fragments showing the highest homology with the probe (see for example band 36 of Fig. 7, which corresponds to the skeletal α-actin gene); and/or to distinct fragments of identical size. In contrast to the case of fractionated DNA, the concentration of specific DNA molecules being much higher, hybridization bands which would be undetectable in total DNA, become evident in individual fractions; (for example, bands 40 and 41 of Fig. 7 are clearly visible in the last two fractions but not in total DNA). Besides, bands corresponding to equal size but different composition were resolved. See, for example, bands 19 and 31, which have maximal intensities in fractions 4 (1.7015 g/cm³) and 8 (1.7060 g/cm³).

c) Some undigested DNA hybridizing to the probe can be seen in the three most (A+T)-rich fractions obtained from the experiment run at 30°C, rT = 0.16 (Fig. 7A); this may be due to some residually bound BAMD blocking EcoRI sites (GAATTTC), which delimit the actin-hybridizing fragments; interestingly this phenomenon is shown by fractions obtained at 30°C, rT = 0.16 (Fig. 7A), but not by those obtained at 20°C, rT = 0.14 (not shown), and parallels the increased buoyant density of (A+T)-rich satellite DNA obtained at high rT values. It should be recalled here that BAMD preferentially binds (A+T)-rich DNA [25].

When a comparison of the three hybridization experiments was done (Table 1), the following points were observed.

a) The majority of hybridization bands were centered in different experiments in fractions having the same modal buoyant density within 1 mg/cm³. Some exceptions to this general rule were due to trivial reasons associated with cutting and pooling of fractions. For instance, band 5 appeared in the lightest 4% of DNA (1.6975 g/cm³) in experiment 2, but in denser fractions (1.6989 g/cm³ and 1.6996 g/cm³; see Fig. 2) in experiments 1 (Fig. 7) and 3 (not shown) respectively; these latter fractions also were the lightest and the second lightest one of the corresponding experiments, but represented 30% and 8% of DNA respectively, and obviously their modal buoyant densities were correspondingly higher.

b) In most cases bands shared narrow distribution indicating that the DNA molecules carrying hybridizable sequences were very homogeneous in base composition. Broader distributions of hybridization bands were, however, also seen (band 17). These could be due to the identical electrophoretic mobility of fragments having different buoyant densities (see above); or to a variable number of BAMD binding sites in the gene-carrying DNA molecules, as generated by random breakage; this may be caused by the fact that the sequence probed was close to the border of isochores endowed with very different base compositions.

c) Some bands were absent in some experiments; this may again be due to trivial reasons, like an incomplete digestion of DNA (see above) or the use of a different probe (the use of the β-actin probe accounts for the lack of hybridization on
Table 1. Buoyant densities of DNA molecules carrying actin-related genes or pseudogenes

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Heavy fractions from experiment 2 of Table 1. Exceptionally the reasons might be fragment length polymorphism; for instance fragment 38, present in experiment 3 but absent in experiment 1, might well correspond to fragments 34, 37 and 41, which have close buoyant densities and are present in experiment 1, but absent in experiment 3, these two experiments having been done on two different DNA preparations (see Table 1).

**CsCl buoyant density of DNA molecules carrying actin-related sequences**

Since the BAMD binding is sequence-specific, the question can be asked whether the molecules detected by the probe in a given Cs2SO4/BAMD fraction have the same buoyant density as the bulk of DNA in the fraction. This is what one would expect on the basis of the regularly increasing modal buoyant densities in CsCl of Cs2SO4/BAMD fractions. An experimental answer to this question was, however, obtained by recentrifuging fractions 3, 4 and 5, from the Cs2SO4/BAMD gradient of Fig. 1a, in preparative CsCl density gradients. The central part and the flanking 10% of each peak from such gradients were then hybridized with the α-actin probe (Fig. 8).

These hybridization experiments showed the following. (a) The intensity of hybridization bands was never higher in the flanks than in the central parts of each fraction, as would be expected if fractionation was associated with a higher or lower BAMD affinity for particular sequences carried by the hybridizing molecules as compared with that of the average molecules present in a given fraction. (b) The higher intensity of hybridization bands in the central part of fraction 4, compared to the flanks, indicated a certain extent of heterogeneity in these fractions, whereas the practically identical intensities of the three parts of fraction 5 indicated a high homogeneity; the case of fraction 3 was an intermediate one.

**DISCUSSION**

**Fractionation of mouse DNA in Cs2SO4/BAMD**

DNA fractionation in Cs2SO4/BAMD density gradients undoubtedly requires further work to be better understood in some of its features, like the multimodal profiles of Figs 1 and 2. At the present stage this technique is, however, very powerful in terms of both resolution and flexibility.

Concerning the resolution, two points should be stressed. The first one is the possibility of combining Cs2SO4/BAMD fractionation with gel electrophoresis to achieve a sort of 'bidimensional' high resolution of DNA fragments. Our experiments on the actin multigene family provide an illustration of what can be obtained. A still higher resolution is, however,
low in fractions only differing in modal buoyant densities by 1.4–1.9 mg/cm³ in the first case, and by 1.7–2.2 mg/cm³ in the second case.

This distribution should be corrected for Brownian diffusion and for concentration effects in order to evaluate the intramolecular compositional heterogeneity. A simple way of taking these correcting factors into consideration is to compare the distribution of DNA molecules carrying single-copy genes with that of intact DNA from phage λ as shown in Fig. 4. This comparison seems to be a fair one in spite of some differences. Indeed, the lower diffusion at 8°C of λ DNA compared to that at 20°C of mouse DNA is largely compensated by the higher molecular size of the latter, 75 kb, compared to that of the former, 49 kb. Otherwise, DNA concentrations in the fractions were very close, as were base compositions and, therefore, BAMD binding. This comparison revealed that the distributions of the gene-carrying mouse DNA molecules and of λ DNA in the Cs₂SO₄/BAMD gradients were extremely close. This indicates that the intramolecular compositional heterogeneity of the former is extremely narrow and very close to that of homogeneous DNA molecules.

This conclusion deserves several comments. (a) Since the mouse DNA molecules carrying the genes under consideration are obtained by a mechanical and enzymatic breakage, which may be assumed to have occurred at random, they are formed by a collection of fragments which correspond to a chromosomal region having a size up to twice that of the molecules, namely 150 kb (since the gene which is probed may be at either end of such molecules). In other words, a high degree of homogeneity is found over a size range reaching 150 kb. (b) This high homogeneity does not need to be a general situation. Indeed, it is conceivable that other genes may be located near the border of isochore characterized by different base composition, a situation which would lead to a higher degree of heterogeneity of the carrier molecules. Alternatively, other genes might be located in regions showing a lesser degree of homogeneity. (c) The results discussed here do not say anything about the compositional heterogeneity of isochore over size ranges beyond 150 kb. Some indirect lines of evidence suggest, however, that the actual size range of homogeneous composition might extend up to the size range of the DNA stretches corresponding to GmcSa and Reverse chromosomal bands [8, 22, 23]. (d) The very low level of compositional heterogeneity of the gene-carrying DNA molecules does not compare with that of the Cs₂SO₄/BAMD fractions in which they were located, as discussed in the following section.

The intramolecular heterogeneity of mouse DNA in the 75–150 kb size range

The high-stringency hybridization experiments of Fig. 6 not only have allowed the localization of three genes, the oncogene c-abl, and the cardiac and skeletal z-actins, but have also provided information of more general interest on the intramolecular heterogeneity of mouse DNA in the 75–150-kb size range. In all cases hybridization overwhelmingly took place in single fractions. These had modal buoyant densities of 1.7022 g/cm³ in the case of the cardiac z-actin gene and of 1.7074 g/cm³ in the case of c-abl and skeletal z-actin genes. The hybridization intensity in the flanking fractions was close to 10% of total intensity (as determined by microdensitometry). In other words the relative amount of the DNA molecules carrying the genes under consideration was very

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The intermolecular heterogeneity of mouse DNA fractions in the size range of 75 kb

The intermolecular (or buoyant density) compositional heterogeneities of Cs₂SO₄/BAMD fractions from mouse DNA have been estimated and are presented in Fig. 9 as H values, calculated according to Schmid and Hearst [26] Band widths were corrected for Brownian diffusion, but no correction was made for concentration effects. Most of the fractions exhibit H values of 1.3–1.5 G + C, the higher values being associated, as expected [8], with fractions approaching 50% G+C. Higher values are found for the first and, more so, for the last fractions, a phenomenon probably due to the increased levels of the lowest-molecular-mass fragments at both ends of the DNA distribution over the gradient, but, also, to
Fig. 9. A comparison of the properties of isolated major components of mouse DNA. Modal buoyant densities (ρ) are given in g/cm³, H as GC % (see [8]). Hatched areas correspond to minor components. Satelite DNA (ρ = 1.691 g/cm³), which represents 7% of total DNA, was not included in the comparison.

a higher heterogeneity in the case of the most (G+C)-rich fraction.

These results should be compared with the heterogeneities exhibited by the gene-carrying DNA molecules, as discussed in the preceding section, and with the DNA components, as isolated in previous work [8]. The first comparison leads to the unequivocal conclusion that the compositional heterogeneity of the fractions is much larger than that of the specific molecules already discussed. This strongly suggests that the heterogeneity of the fractions is the result of a large overlap of DNA molecules endowed with much narrower distributions.

A comparison of the fractions from the Cs₂SO₄/BAMD density gradient with the major components of the mouse genome [8] shows that the intermolecular heterogeneity of the former is on average about one-half of the latter. If bacterial DNAs having the same base composition and the same molecular mass are taken as references, the heterogeneities of the fractions and of the components are lower and higher, by 25–50%, respectively. A direct implication of the lower heterogeneity of fractions compared to components is that a gene localization is better defined in the first case than in the second. As already mentioned, an even better definition could be achieved if the gradient was cut in a larger number of fractions.

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