J. Mol. Biol. (1976) **108**, 219–235

An Analysis of Eukaryotic Genomes by Density Gradient Centrifugation

JEAN-PAUL THIERY†, GABRIEL MACAYA AND GIORGIO BERNARDI

Laboratoire de Génétique Moléculaire, Institut de Biologie Moléculaire Paris 75005, France

(Received 6 February 1976, and in revised form 21 June 1976)

DNA preparations from 25 eukaryotes, ranging from yeast to man, were analyzed in their base composition, sedimentation coefficient, modal and mean buoyant density in CsCl.

Four mammalian and two amphibian DNAs were fractionated by preparative Cs_2SO_4–Ag^+ density gradient centrifugation. The CsCl band profile of each fraction was analyzed in terms of Gaussian curves; this allowed the buoyant densities and the relative amounts of the components present in each DNA to be assessed.

Direct analysis of the CsCl band profiles of unfractionated DNA showed a satisfactory agreement with the results obtained in the combined Cs_2SO_4–Ag^+, CsCl investigations. This simpler procedure was therefore applied to all 25 eukaryotic DNAs.

The main finding of this work is the recognition of phylogenetic differences at the macromolecular level in the organization of eukaryotic genomes. The differences concerning the main band DNA are: (a) the three major components (1·697, 1·704 and 1·709 g/cm^3) first observed in the main band of calf DNA by Filipski *et al.* (1973) were also found in the other ten mammalian DNAs investigated here; similar components appear to be present in avian genomes; in both cases, the 1·704 and 1·709 g/cm^3 components are responsible for the skewness to the heavy side of the CsCl main band of these DNAs; (b) the DNAs of reptiles, amphibians and fish showed a much lower and decreasing skewness of their CsCl bands compared to both mammalian and avian DNAs; the Cs_2SO_4–Ag^+, CsCl combined analysis of two amphibian DNAs revealed discrete components, different from those of mammals and birds; (c) essentially symmetrical bands in CsCl were exhibited by three invertebrate DNAs; (d) the DNAs from three unicellular eukaryotes exhibited perfectly symmetrical bands in CsCl and could not be resolved into discrete components.

A number of observations on minor and satellite DNA components collected in this work are reported.

1. Introduction

It is well known since the classical work of Meselson *et al.* (1957) that, when centrifuged to equilibrium in CsCl density gradients, bacterial DNAs show unimodal, symmetrical bands, whereas calf thymus DNA exhibits a multimodal, broad, asymmetrical band. A number of theoretical and experimental investigations on equilibrium centrifugation in density gradients have mostly dealt with the simple case of

† Present address: The Rockefeller University, New York, N.Y. U.S.A.

uniform populations of DNA molecules such as those of bacteriophage DNAs (Schmid & Hearst, 1969,1971,1972). Early attempts to study the much more complex case of animal DNAs (Sueoka, 1959,1961,1962) have not been followed up.

Three years ago we reported (Filipski *et al.*, 1973) that the main band of calf DNA, M_w† $= 5\times10^6$ to 7×10^6, was not formed by genome fragments having a continuous distribution of their average G + C content, as it was commonly believed, but by three distinct families of fragments. These were characterized by buoyant densities in CsCl equal to 1·697, 1·704 and 1·709 g/cm^3, and represented about 50, 25 and 10% of the DNA, respectively. These major DNA components could be separated from each other by preparative centrifugation in Cs_2SO_4–Ag^+ density gradients; they were characterized by symmetrical CsCl bands which exhibited standard deviations close to those of bacterial DNAs of comparable molecular weight; these components were responsible for the asymmetry of the CsCl main band of calf DNA.

Three important questions were raised by the presence of these major DNA components in the calf genome: their evolutionary origin, their relative arrangement, and their intramolecular heterogeneity. In this paper, we investigated the first question by studying a number of eukaryotic DNAs by centrifugation to equilibrium in density gradients of Cs salts. The accompanying paper (Macaya *et al.*, 1976*b*) considers the other two points.

Our experimental approach can be outlined as follows. (1) A total of 25 eukaryotic DNAs, characterized in their sedimentation coefficients and G + C contents, were centrifuged to equilibrium in CsCl, and their modal and mean buoyant densities, ρ_0 and $\langle\rho\rangle$, were determined. This provided some basic information on the DNAs. (2) Six eukaryotic DNAs were fractionated by preparative Cs_2SO_4–Ag^+ density gradient centrifugation. Owing to a differential binding of Ag^+ ions, this procedure can resolve not only a number of apparent and cryptic satellite‡ DNAs (Corneo *et al.*, 1968*b*), but also several minor DNA components and the major components forming the main band of calf thymus DNA (Filipski *et al.*, 1973). The 15 to 25 fractions so obtained from each DNA were then analyzed in their sedimentation coefficients and centrifuged to equilibrium in analytical CsCl density gradients. The band profiles were resolved into a number of Gaussian curves and the corresponding DNA components were defined in terms of their buoyant densities and relative amounts in the DNAs analyzed. (3) A direct resolution into these Gaussian components of CsCl band profiles of unfractionated DNAs was also made, and shown to be in satisfactory agreement with the results obtained in the more detailed investigations involving preliminary Cs_2SO_4–Ag^+ centrifugation. All 25 eukaryotic DNAs were studied by this simpler procedure.

2. Materials and Methods

(a) *DNA preparations*

Animal tissues and their source were the following: human placenta, Hôpital International de la Cité Universitaire, Paris; human leucocytes, Centre de Transfusin Sanguine, Hôpital Cochin, Paris; cat and dog livers, Ecole Nationale Vétérinaire, Maisons-Alfort, France; guinea pigs, chinese hamsters, Wistar rats, C_3H and Balb-c mice, and albino rabbits, the animal house of our Institute. Garden dormouse (*Glis glis*), Normandy,

† M_w is the weight average molecular weight.

‡ For the sake of convenience we will distinguish here minor and satellite components, the latter corresponding to simpler sequence or highly repetitive DNAs.

France; hedgehog (*Erinaceus* sp.), Massif Central, France; sea gull (*Larus argentatus*), Zoology Laboratory of the University of Paris VII; chicken embryo (*Gallus* sp.), Dr F. Chapeville; *Iguana iguana* erythrocytes, Dr Gamez, Agronomy School, San José de Costa Rica; *Pleurodeles waltlii* testes, Professor Lacroix, Embryology Department, University of Paris VI; *Testudo graeca*, a local fishery; *Opsanus tau* liver, Professor A. E. V. Haschemeyer, Hunter College, New York; *Paracentrotus lividus*, Laboratoire de Biologie Marine, Banyuls, France. DNA preparations from these tissues were made according to Kay *et al.* (1952) and further purified by chromatography on hydroxyapatite in the presence of 3 M-KCl (Filipski *et al.*, 1973).

DNAs from calf thymus and liver were preparations already described (Filipski *et al.*, 1973). DNA from salmon (*Salmo salar*) testes was obtained from Worthington, Freehold, N. J. DNAs from *Xenopus laevis* erythrocytes, *Drosophila melanogaster* embryos, *Strongylocentrotus purpuratus* sperm and *Tetrahymena pyriformis* macronuclei were the gifts of Drs I. B. Dawid, J. E. Hearst, R. J. Britten and L. Marcaud, respectively. Nuclear DNA from *Euglena gracilis* was obtained by chromatography on hydroxyapatite of total nucleic acids (Stutz & Bernardi, 1972). *Saccharomyces cerevisiae* DNA was prepared from wild-type strain B (Bernardi *et al.*, 1970) according to a procedure to be published elsewhere.

(b) *Analytical density gradient centrifugation in CsCl*

DNA solutions in 0·005 M-NaCl, 0·01 M-Tris (pH 7·8), were made about 1·700 g/cm^3 in density by addition of solid CsCl (Suprapur, Merck, Darmstadt, Germany). Centrifugations to equilibrium were done at 25°C using a 6-hole titanium An-G rotor, 12 mm double-sector Kel-F cells and a Spinco model E ultracentrifuge equipped with a monochromator, a photoelectric scanner and a multiplexer; a concave mirror replaced the upper plane mirror and the camera lenses. In the most recent work a modification of the multiplexer system (G. Macaya and M. Grosjean, manuscript in preparation) permitted the simultaneous study of 9 DNA samples.

Equilibrium was judged to be reached when the variance of the profile decreased by less than 1% in 1 h. Scanning was done in the 265 to 280 nm wavelength range using a photomultiplier slit-width of 0·06 or 0·12 mm. Scanning speeds of 0·25 to 1·25 cm/min were used with a chart speed of 6 to 30 cm/min; this corresponds to enlargements of 24 to 114-fold along the abscissa.

In order to calculate the buoyant density, ρ, at any point of abscissa r, from the rotation axis, the relationship

$$\rho = \rho\kappa - \frac{\omega^2}{2\,\beta_0}(r_\kappa^2 - r^2) \tag{1}$$

was used, where the subscript κ refers to the marker, ω is the angular velocity in radians s^{-1}, and β_0 was taken as equal to $1{\cdot}19 \times 10^9$ cm^5 g^{-1} s^{-2} (Ifft *et al.*, 1961). Under such conditions, using phage 2C DNA (ρ = 1·742 g/cm^3; Szybalski, 1968) as a density marker, a reproducible modal buoyant density, ρ_0 (density at the peak maximum, located at a distance r_0 from the rotation axis), of 1·7103 g/cm^3 was obtained for *E. coli* DNA.

The mean buoyant density, $\langle\rho\rangle$, was calculated from the first moment of the band profile about the center of rotation:

$$\langle r\rangle = \frac{\int_0^\infty cr\,\mathrm{d}r}{\int_0^\infty c\,\mathrm{d}r}, \tag{2}$$

and from eqn (1), c being the DNA concentration at point of abscissa r.

The variance of the profile, $\langle\delta^2\rangle$, is equal to the second moment about the mean

$$\langle\delta^2\rangle = m_2 = \frac{\int_{\mathrm{BAND}} c\delta^2\,d\delta}{\int_{\mathrm{BAND}} c\,d\delta}, \tag{3}$$

with $\delta = r - \langle r\rangle$.

Integrals of eqns (2) and (3) were calculated using Simpson's rule, with radial steps corresponding to 0·04 or 0·08 mm in cell dimensions.

(c) *Analysis of CsCl band profiles*

Resolution of the CsCl profiles was done on a Dupont de Nemours (Wilmington, Del.) curve resolver model 310, equipped with 10 channels and a recorder, using either 3-fold photographic enlargements of the scans having a 24-fold magnification, or large scans having a 114-fold enlargement (these scans had a 1·82 m length, reference to reference). Band profiles used for such analyses had A_{265nm} values at the peak comprised between 0·6 and 1·0; this permitted us to properly evaluate the amounts of the components and to minimize differential concentration effects.

(d) *Preparative density gradient centrifugation*

Preparative centrifugation to equilibrium in Cs_2SO_4–Ag^+ density gradients was performed as previously described (Filipski *et al.*, 1973) using a Spinco L2-65B centrifuge and a type 30 rotor at a temperature of 25°C. The criteria for the choice of experimental conditions were the following. Since the resolution of DNA components in Cs_2SO_4–Ag^+ density gradient centrifugation depends upon pH and r ratio (the molar Ag^+/DNA-P ratio), preliminary experiments were done, usually in the analytical ultracentrifuge, to assess the influence of these 2 parameters on the band profiles of the DNAs investigated. The ranges explored were 7·8 to 10·3 for pH, and 0·2 to 0·4 for the r ratio, the choice of these ranges being discussed elsewhere (manuscript in preparation). Experimental conditions (pH, r, slope of the Cs_2SO_4 density gradient, load) giving the maximum spread and skewness of the main band were chosen, regardless of the resolution of satellite bands.

(e) *Other methods*

Sedimentation velocity experiments were done by the band centrifugation method, with Vinograd 30-mm, single-sector cells (Prunell & Bernardi, 1973). Base composition was determined, after enzymatic digestion to nucleosides, on Aminex A6 columns (Thiery *et al.*, 1973).

3. Results

(a) *CsCl band analysis and G + C contents of eukaryotic DNAs*

The modal and mean buoyant densities in CsCl, ρ_0 and $\langle\rho\rangle$, of all DNAs investigated in the present work are given in Table 1, which also presents the base compositions and the sedimentation coefficients of the DNA preparations.

TABLE 1

Properties of eukaryotic DNAs

DNA source	Tissue	$s_{20,w}$	G + C (%)	ρ_0† (%)	$\langle\rho\rangle$† (g/cm³)	$\langle\rho\rangle - \rho_0$ (mg/cm³)
ANIMALIA‡						
I CHORDATA						
(a) MAMMALIA						
Homo sapiens (man)	Leucocytes	25		1·6985	1·7008	2·3
	Placenta	22·3	40·3	1·6990	1·7010	2·0
Bos taurus (calf)	Liver	25·2		1·7000	1·7039	3·9
	Thymus	26	43·2	1·6997	1·7033	3·6
Felis domesticus (cat)	Liver	32·7	42·8	1·7002	1·7031	2·9
Canis familiaris (dog)	Liver	31·3	44·1	1·7005	1·7040	3·5
Cavia porcellus (guinea pig)	Liver	24·8	40·1	1·6982	1·7010	2·8

TABLE 1—continued

DNA source	Tissue	$s_{20,w}$	G + C (%)	ρ_0† (g/cm³)	$\langle\rho\rangle$† (g/cm³)	$\langle\rho\rangle - \rho_0$ (mg/cm³)
Cricetus norvegicus (Chinese hamster)	Liver	22·6	42·8	1·7000	1·7015	1·5
Rattus sp. (rat)	Liver	27·6	41·8	1·7006	1·7021	1·5
Mus musculus (mouse)	Liver	24·5	40·3	1·7007	1·7008	0·1
					1·7020	1·3§
	Thymus	21·6		1·7008	1·7016	0·8
Glis glis (garden dormouse)	Liver	35·8	40·3	1·6991	1·7004	1·3
Oryctolagus cuniculus (rabbit)	Liver	18·6	44·5	1·6999	1·7033	3·4
Erinaceus sp. (hedgehog)	Thymus	32·8	41·9	1·7004	1·7035	3·1
(b) AVES						
Larus argentatus (sea-gull)	Liver	23·7	47	1·6998	1·7031	3·3
Gallus sp. (chicken)	Embryo	20	45	1·7001	1·7031	3·0
(c) REPTILIA						
Iguana iguana	Erythrocytes	20·5	43·9	1·7015	1·7022	0·7
Testudo graeca (Greek turtle)	Liver	25·1	46	1·7027	1·7042	1·5
(d) AMPHIBIA						
Pleurodeles waltlii	Testes	27·1	46·5	1·7041	1·7047	0·6
Xenopus laevis	Erythrocytes	35	40·9	1·6991	1·6997	0·6
(e) PISCES						
Opsanus tau (toadfish)	Liver	20	42	1·7002	1·7004	0·2
Salmo salar (salmon)	Testes	20·5	43·5	1·7028	1·7035	0·7
II ECHINODERMATA						
(a) ECHINOIDEA						
Strongylocentrotus purpuratus	Sperm	17	36·6	1·6989	1·6995	0·6
Paracentrotus lividus	Sperm	19	35·4	1·6972	1·6974	0·2
III ARTHROPODA						
(a) INSECTA						
Drosophila melanogaster	Embryo	27·4	39·1	1·7025	1·7014	−1·1
FUNGI						
ASCOMYCOTA						
Saccharomyces cerevisiae	Total	28	37·1	1·6995	1·6984	−1·1§
			40¶		1·7001	0·6§
PROTISTA						
I EUGLENOPHYTA						
Euglena gracilis	Nuclear	16	49·9	1·7080	1·7080	0
II CILIOPHORA						
Tetrahymena pyriformis	Macro nuclei	17·7	29·7	1·6910	1·6910	0
MONERA						
EUBACTERIAE						
Escherichia coli		34	51	1·7105	1·7101	−0·4

† The error on the ρ_0 values is $\pm 0{\cdot}0005$ g/cm³; that on the $\langle\rho\rangle$ values is $\pm 0{\cdot}0002$ g/cm³.

‡ Systematic nomenclature according to Whittaker (1969).

§ $\langle\rho\rangle$ and $\langle\rho\rangle - \rho_0$ values calculated for main band DNA, not taking into account the contribution of apparent satellites. See also the text.

¶ DNA free of mitochondrial DNA and of the 1·705 g/cm³ satellite.

The ρ_0 values of mammalian and avian DNAs were found to be very close to 1·700 g/cm^3. A wider range of ρ_0 values was found in the other vertebrates (1·699 to 1·704 g/cm^3) and in invertebrates (1·697 to 1·702 g/cm^3). A much wider range was found in the unicellular eukaryotes investigated (1·691 to 1·708 g/cm^3).

The $\langle\rho\rangle - \rho_0$ values characterize fairly well the CsCl band profiles; they are a measure of the asymmetry of the peak and are positive or negative, according to whether the peak is skewed on the heavy or the light side. This asymmetry is due to the presence of apparent or cryptic satellite bands and/or to an asymmetry of the main band; obviously, these effects are additive. Calf DNA is an example of the first possibility with its very high $\langle\rho\rangle - \rho_0$ value being largely due to its heavy satellites. A real asymmetry of the main band also contributes to the high $\langle\rho\rangle - \rho_0$ value of mammalian and avian DNAs as can be seen in DNAs without heavy satellites, like

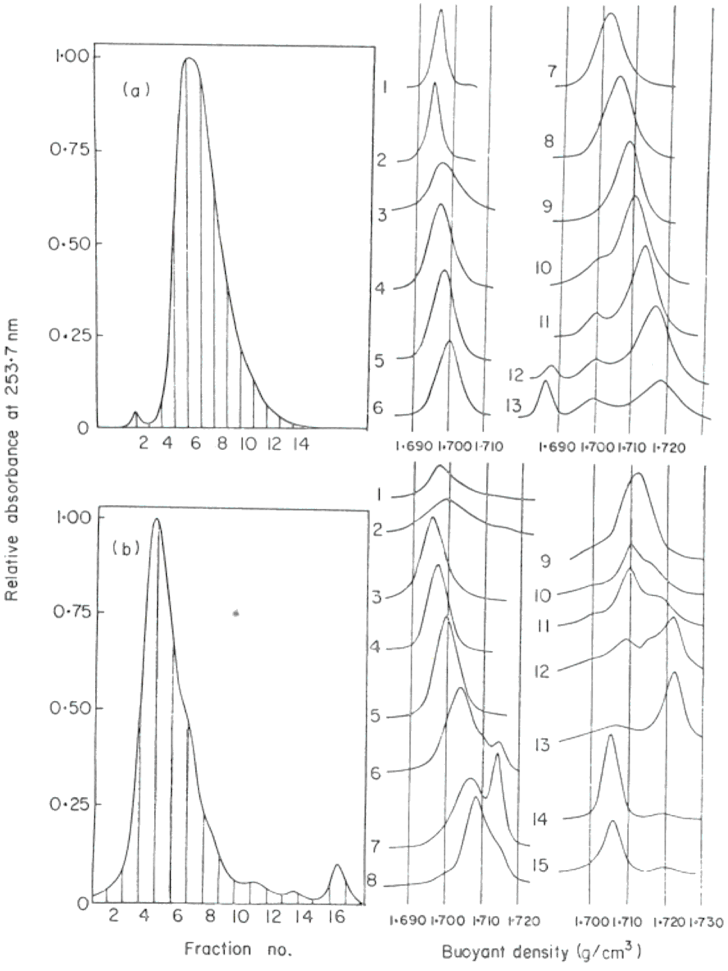

FIG. 1. Analysis of man and calf DNA components in Cs_2SO_4–Ag^+ density gradient.
(a) Human placenta DNA ($A_{260nm} = 0{\cdot}86$) in borate buffer (pH 9·2) containing $AgNO_3$ ($r = 0{\cdot}32$) and Cs_2SO_4 ($\rho = 1{\cdot}48$ g/cm^3) was centrifuged at 25,000 revs/min for 90 h.
(b) Calf thymus DNA ($A_{260nm} = 0{\cdot}54$) in borate buffer (pH 9·2) containing $AgNO_3$ ($r = 0{\cdot}35$) and Cs_2SO_4 ($\rho = 1{\cdot}495$ g/cm^3) was centrifuged as described above.

those of mouse and man. Such main band asymmetry decreases in DNAs from reptiles, amphibia, fish and invertebrates. The main bands of unicellular eukaryotes are perfectly symmetrical. Interestingly, *E. coli* DNA exhibits a slightly negative value of $\langle\rho\rangle - \rho_0$.

(b) *Fractionation of eukaryotic DNAs in preparative Cs_2SO_4–Ag^+ density gradients, and CsCl analysis of the fractions*

Six eukaryotic DNAs were fractionated in preparative Cs_2SO_4–Ag^+ density gradients; 15 to 25 fractions were collected and analyzed by sedimentation velocity and by centrifugation in CsCl density gradients. Figures 1 to 3 show the results obtained for human, calf, mouse, guinea pig, *Xenopus*, and *Pleurodeles* DNAs. The analysis of the CsCl band profiles of Cs_2SO_4–Ag^+ fractions in terms of Gaussian

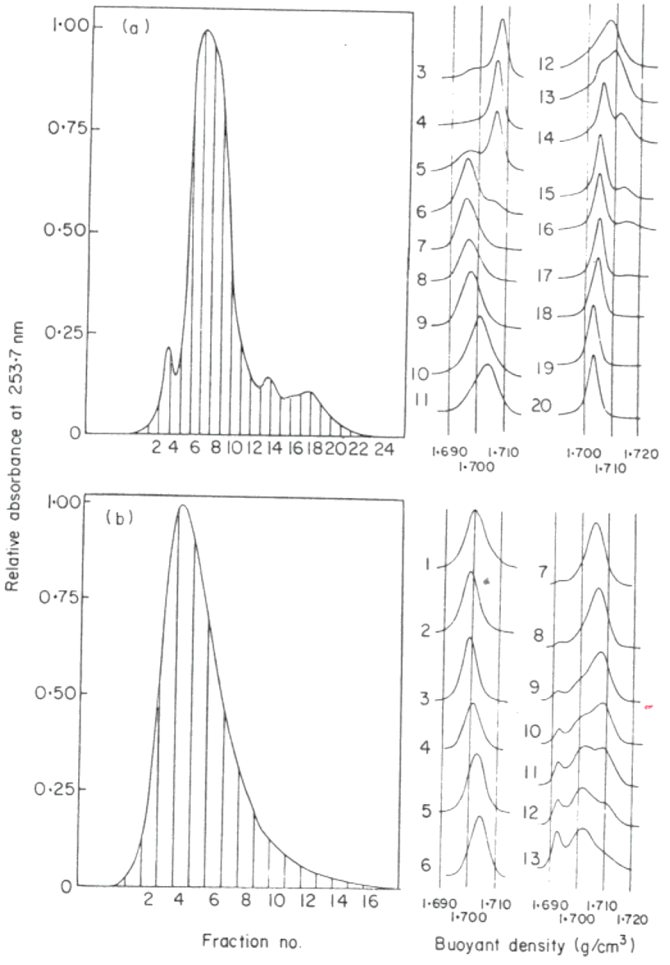

FIG. 2. Analysis of guinea pig and mouse DNA components in Cs_2SO_4–Ag^+ density gradient.
(a) Guinea pig DNA ($A_{260nm} = 1{\cdot}80$) in borate buffer (pH 9·2) containing $AgNO_3$ ($r = 0{\cdot}27$) and Cs_2SO_4 ($\rho = 1{\cdot}49$ g/cm³) was centrifuged at 27,000 revs/min for 100 h at 25°C.
(b) Mouse liver DNA ($A_{260nm} = 0{\cdot}5$) in borate buffer (pH 9·2) containing $AgNO_3$ ($r = 0{\cdot}32$) and Cs_2SO_4 ($\rho = 1{\cdot}50$ g/cm³) was centrifuged at 25,000 revs/min for 96 h.

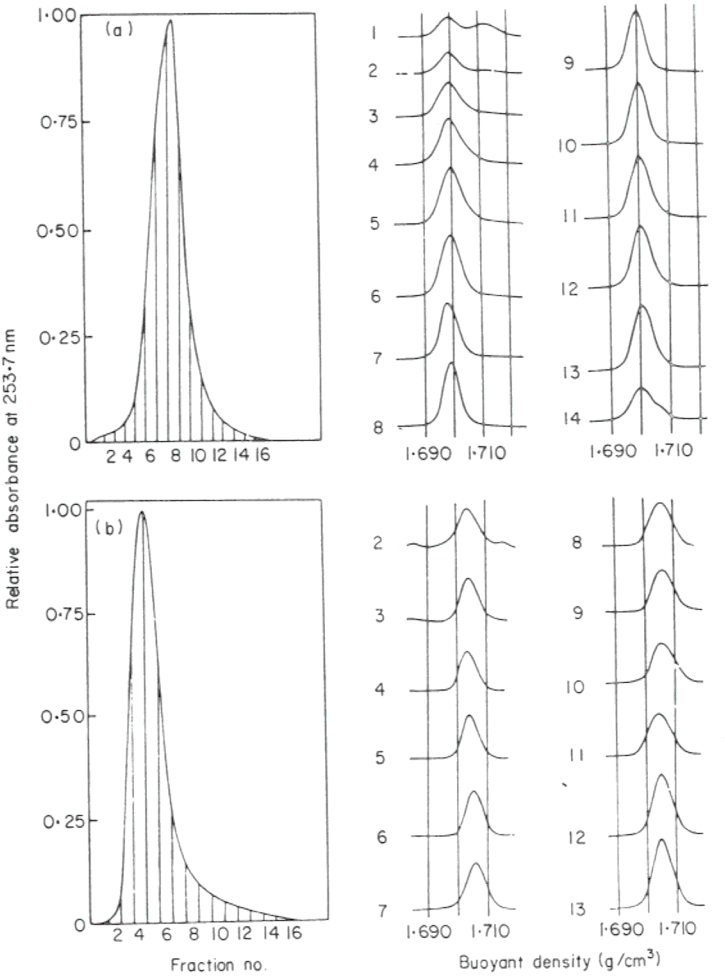

FIG. 3. Analysis of *Xenopus* and *Pleurodeles* DNA components in Cs_2SO_4–Ag^+ density gradient.
(a) *Xenopus* erythrocytes DNA ($A_{260nm} = 0{\cdot}46$) in borate buffer (pH 9·2) containing $AgNO_3$ ($r = 0{\cdot}35$) and Cs_2SO_4 ($\rho = 1{\cdot}50$ g/cm³) was centrifuged as described for mouse liver DNA.
(b) *Pleurodeles* testes DNA ($A_{260nm} = 0{\cdot}51$) in borate buffer (pH 9·2) containing $AgNO_3$ ($r = 0{\cdot}35$) and Cs_2SO_4 ($\rho = 1{\cdot}50$ g/cm³) was centrifuged as described above.

curves has been done as described in the accompanying paper (Macaya *et al.*, 1976, Appendix, section (a)). The results obtained have been used to estimate the amounts and buoyant densities of the components present in each DNA (Table 2).

DNA components have been divided into three descriptive classes: (1) major components, each representing, as a rule, at least 10% of total DNA; of these a main component, forming 50% or more of the genome, should be specially noted, and will be frequently referred to; (2) minor components, each of which could form up to 3% of total DNA; (3) satellite components; these could be easily distinguished from minor components because they characteristically show hypersharp peaks in CsCl density gradients (accompanying paper; Appendix, section (c)); in addition, satellite components were already known in most cases, in both their buoyant densities and approximate amounts.

TABLE 2

Component patterns of eukaryotic DNAs (combined Cs_2SO_4–Ag^+ and CsCl analysis)

DNA source Species	Tissue	Major components (ρ)	(%)	Minor components (ρ)	(%)	Satellites (ρ)	(%)
Man	Placenta	1·697	16 } 65	1·712	0·7	1·6875	0·15
		1·699	49 }	1·7135	1·5	1·6955	1·2
		1·704	18	1·716	1·3	1·6995	0·5
		1·709	11	1·718	0·3		
Calf	Thymus	1·697	25 } 57	1·712	2·5	1·705	4
		1·700	32 }	1·714	0·15	1·710	3
		1·704	15	1·715	2	1·715	7
		1·709	9	1·719	0·1	1·723	0·5
Guinea pig	Liver	1·696	45 } 69	1·697	0·1	1·704	5
		1·7005	24 }	1·712	2	1·7055	5
		1·7045	8	1·715	0·7	1·707	5
		1·7085	4				
Mouse	Liver	1·699	39 } 61	1·699	2·8	1·691	7
		1·7015	22 }	1·704	2		
		1·7045	21	1·712	0·15		
		1·709	8				
X. laevis	Erythrocytes	1·699	70	1·705†	3	1·712	0·3
		1·7005	21	1·705†	0·2		
		1·706	5				
P. waltlii	Testes	1·704	65			1·686	0·4
		1·705	9			1·7165	0·2
		1·706	25				

† The first 1·705 g/cm³ component is present in fractions 3 to 5; the second 1·705 g/cm³ component is present in fractions 14 to 16 (Fig. 4).

(i) *Mammalian DNAs*

DNAs from man, calf, mouse and guinea pig are characterized by the presence of three major components: (1) a main component forming 57 to 65% of total DNA; this component is formed by two sub-components whose relative amounts and buoyant densities slightly vary from one species to another, as shown in Table 2; (2) two major components banding at 1·704 and 1·709 g/cm³, respectively; these components form 15 to 20% and about 10%, respectively, of total DNA and are mainly responsible for the typical skewness on the heavy side of the CsCl bands of mammalian DNAs. It should be noted that the guinea pig genome appears to have a larger percentage of the main component (69%) and lower percentages of the other major components (8% and 4%, respectively) compared to the other mammalian DNAs.

Although our attention here was focused on the major components of mammalian DNAs, a number of minor components were characterized in their buoyant densities and amounts. Ribosomal genes and mitochondrial DNA may correspond to some of these components. A discussion of the rather complex case of minor components of human DNA is presented elsewhere (Macaya *et al.*, 1976*a*).

As far as satellite components are concerned, our data on the buoyant densities and amounts are sometimes at a slight variance with previously published values. A more serious discrepancy with the literature concerns the human satellites, since we could only detect three out of the four reported so far. The 1·6875 g/cm^3 component (Fig. 1, fractions 12 and 13) corresponds to satellite I (1·687 g/cm^3) of Corneo *et al.* (1967,1968*a*) and Schildkraut & Maio (1969); the 1·6995 g/cm^3 component (Fig. 1, fractions 10 to 13) corresponds to satellite IV (1·700 g/cm^3) of Corneo *et al.* (1972). Satellites I and IV were found here, as by Corneo, on the light side of the Cs_2SO_4–Ag^+ density gradient ($r = 0{\cdot}2$ to $0{\cdot}3$). The 1·695 g/cm^3 component (Fig. 1, fractions 1 and 2) had a density close to that reported by Corneo (1·696 g/cm^3) for his satellite III (Corneo *et al.*, 1971), yet its behaviour in density gradients is similar to that of satellite II (1·693 g/cm^3; Corneo *et al.*, 1970) in that the 1·695 g/cm^3 components was found here on the heavy side of the main peak and not on the light one, at r values comprised between 0·2 and 0·32. A further analysis of both sides of the peak in Cs_2SO_4–Ag^+ failed to reveal any 1·693 g/cm^3 component. Under these circumstances, we conclude that satellites II and III of Corneo may be the same satellite.

(ii) *Amphibian DNAs*

The DNA component patterns of *X. laevis* and *P. waltlii* were very different from each other and from those of mammalian DNAs. The *X. laevis* DNA was characterized by three major components having buoyant densities equal to 1·699, 1·7005 and 1·706 g/cm^3 and representing 70%, 21% and 5% of the genome, respectively. The *Pleurodeles* DNA was characterized by three components of densities 1·704, 1·705 and 1·706 g/cm^3, representing 65%, 9% and 25% of the genome, respectively. It should be noted that the latter DNA is different from the other vertebrate DNAs examined here in two respects: it has an exceptionally high *c*-value (22·5 pg of DNA per haploid cell; Becak *et al.*, 1970) and a buoyant density much higher than the average of 1·700 g/cm^3 (Table 1).

In *X. laevis* DNA two minor components having the same buoyant density in CsCl (1·705 g/cm^3), but originating from the opposite sides of the Cs_2SO_4–Ag^+ peak were detected. The one representing only 0·2% of total DNA and deriving from the light side of the Cs_2SO_4–Ag^+ (Fig. 3, fraction 14) can be identified with mitochondrial DNA (Birnstiel *et al.*, 1968). A satellite DNA having a density of 1·712 g/cm^3 was also observed in *X. laevis* DNA (Fig. 3, fraction 1); in contrast, a component of density 1·723 g/cm^3, corresponding to ribosomal DNA (Sinclair & Brown, 1971) was not detected, probably because of its small amount, 0·2%, in the genome (Brown & Sugimoto, 1974) and its behaviour in the density gradient. In *P. waltlii* DNA two small satellites banded at 1·686 g/cm^3 and 1·716 g/cm^3 (Fig. 3, fraction 2); similar components were already observed in *Triturus viridescens* (Barsacchi & Gall, 1972), in which case the heavier one was identified with ribosomal DNA.

(c) *CsCl band patterns shown by eukaryotic DNAs*

The results obtained by using the curve resolver directly on unfractionated DNA were compared with those obtained after fractionation in Cs_2SO_4–Ag^+ and CsCl as in the previous sections. Even in the relatively complicated cases of mammalian genomes, a fairly satisfactory correspondence was obtained for both the buoyant densities and relative amounts of DNA components. This encouraged us to use the

more direct approach, which clearly is much less laborious, on a larger number of eukaryotic genomes.

Results obtained with 11 mammalian DNAs are shown in Figure 4. In each, the main component forms 50 to 65% of the genome; they are present as two sub-components in the case of man, calf thymus, guinea pig, mouse and garden dormouse, and as a single component in all other cases. The relative amounts of the two sub-components vary in the DNAs from the two different human tissues; in the case of calf tissues, a single component, banding at 1·699 g/cm^3 is present in liver DNA and two sub-components, banding at 1·697 and 1·700 g/cm^3, respectively, are present in calf thymus. The other major components banding at 1·704 and 1·709 g/cm^3 are characteristically present in all DNAs investigated; in all cases, they appear to be responsible for the skewness on the heavy side of the CsCl profiles.

The two avian DNAs (Fig. 5) show major components similar to those of mammalian DNAs; a slight skewness on the heavy side is also evident in the DNAs from the two reptiles investigated.

The situation is quite different in the case of the DNAs from other vertebrates and

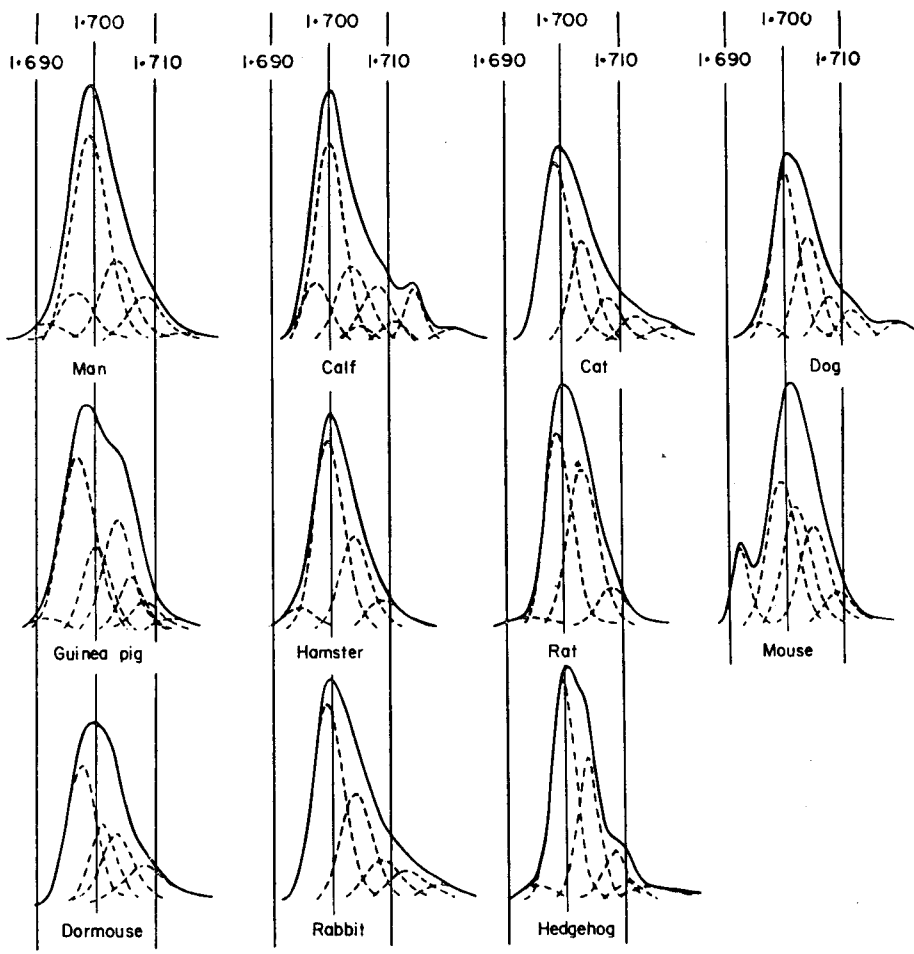

FIG. 4. CsCl analysis of DNAs from 11 mammals.

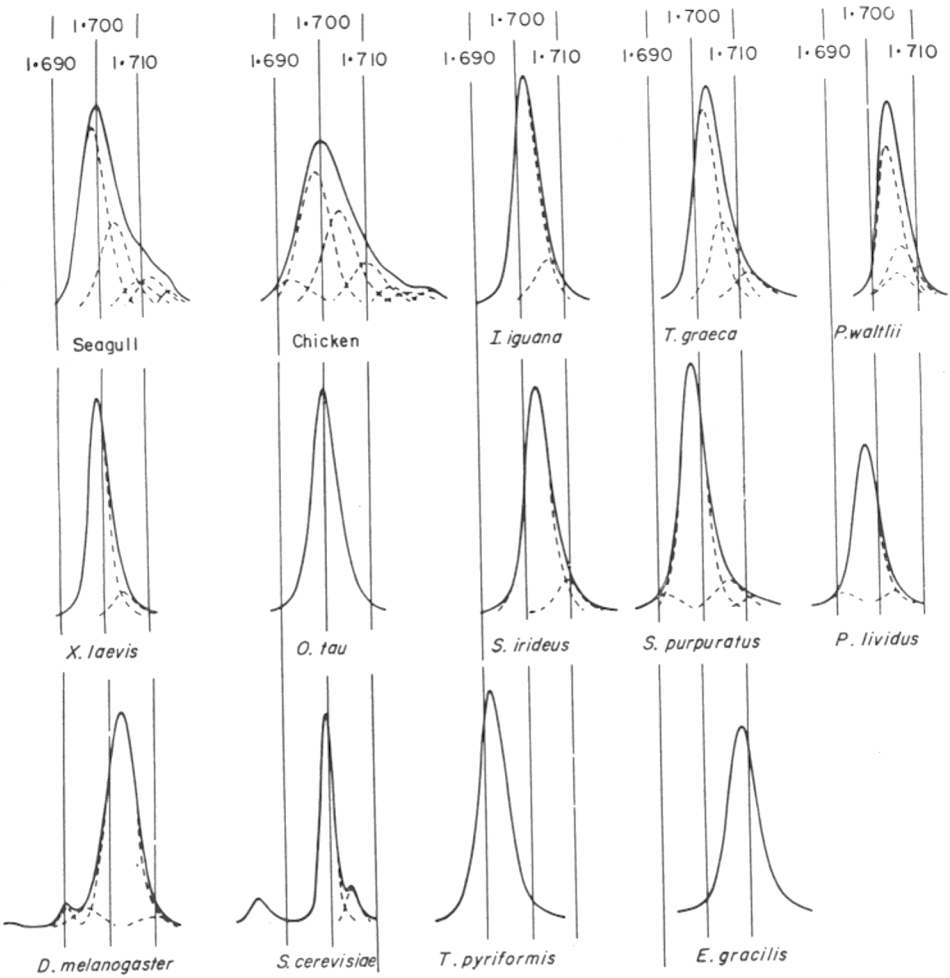

FIG. 5. CsCl analysis of DNAs from birds, reptiles, amphibia, fishes, invertebrates and unicellular eukaryotes.

invertebrates. If a generalization can be made on the basis of the limited number of species investigated, these genomes are characterized by the fact that the components banding at 1·697 to 1·702 g/cm^3 form more than 80% of the genome causing the CsCl band to be almost symmetrical (Fig. 5). A peculiar situation is found, as already reported above, in *Pleurodeles* DNA; in this case the main component had a density of 1·704 g/cm^3.

Perfectly symmetrical main bands, of different buoyant densities, were found in the three unicellular eukaryotes studied.

As expected, a lower amount of information was obtained by the direct analysis of CsCl bands compared to the combined Cs_2SO_4–Ag^+, CsCl approach. A comparison of the results of Tables 2 and 3 for *P. waltlii* and *X. laevis* is quite significant in this respect. Such limitations become more serious as far as minor and satellite components are concerned; for this reason these were not distinguished from each other in Table 3.

TABLE 3

Component patterns of eukaryotic DNAs (CsCl analysis)

DNA source		Major components		Minor components and satellites	
Species	Tissues	(ρ)	(%)	(ρ)	(%)
Man	Leucocytes	1·697	27 } 66	1·693	3
		1·699	39 }	1·713	2
		1·704	19		
		1·709	9		
	Placenta	1·697	13 } 65	1·692	3
		1·699	52 }	1·716	1
		1·704	19		
		1·709	11		
Calf	Liver	1·699	50	1·705	4
		1·704	20	1·710	3
		1·709	13	1·716	7
				1·723	1
	Thymus	1·697	15 } 53	1·705	4
		1·700	38 }	1·711	3
		1·704	21	1·716	7
		1·709	13	1·723	1
Cat	Liver	1·699	55	1·7125	6
		1·704	24	1·719	3
		1·709	10		
Dog	Liver	1·700	52	1·694	3
		1·704	23	1·713	6
		1·709	10	1·719	1
				1·722	3
Guinea pig	Liver	1·6965	44	1·691	2
		1·7005	22	1·7065	15
		1·7045	8	1·714	2
		1·709	8		
Chinese hamster	Liver	1·699	63		
		1·704	26	1·694	2
		1·709	8		
Rat		1·699	49		
		1·704	39	1·693	2
		1·709	9		
Mouse	Liver	1·699	36 } 60		
		1·7015	24 }	1·6915	7
		1·705	24	1·695	3
		1·709	6		
	Thymus	1·699	35 } 61		
		1·7015	26 }	1·6915	7
		1·7045	24	1·695	2
		1·709	5	1·713	1
Garden dormouse	Liver	1·698	42 } 63		
		1·701	21 }		
		1·7045	22		
		1·709	15		
Rabbit	Liver	1·699	55	1·7135	7
		1·704	26	1·719	2
		1·709	10		
Hedgehog	Thymus	1·6995	53	1·695	2
		1·7045	29	1·7125	3
		1·710	10	1·718	2
Sea-gull	Liver	1·699	61	1·712	7
		1·704	24	1·716	2
		1·709	7		

TABLE 3—continued

DNA source Species	Tissue	Major components (ρ)	(%)	Minor components and satellites (ρ)	(%)
Chicken	Embryo	1·699	47	1·694	6
		1·704	28	1·714	3
		1·709	12	1·723	2
I. iguana	Erythrocytes	1·7015	85		
		1·706	15		
T. graeca	Liver	1·702	64	1·711	5
		1·706	27	1·714	4
P. waltlii	Testes	1·7036	85	1·706	7
				1·709	9
X. laevis	Erythrocytes	1·699	92		
		1·704	8		
S. salar	Sperm	1·7028	93	1·709	7
O. tau	Liver	1·699	100		
S. purpuratus	Sperm	1·698	82	1·692	6
				1·7055	9
				1·712	3
P. lividus	Sperm	1·697	89	1·6995	4
				1·7035	7
D. melanogaster	Embryos	1·7023	96	1·676	1
				1·690	3
S. cerevisiae	Total	1·684	11		
		1·6995	79		
		1·705	10		
E. gracilis	Nuclear	1·708	100		
T. pyriformis	Macro nuclei	1·691	100		
E. coli		1·710	100		

4. Discussion

(a) *Mammalian DNAs*

The main component of mammalian DNAs represents 50 to 65% of total DNA. The variable amounts of minor and satellite components in mammalian DNAs may be largely responsible for this rather wide range of relative amounts of the main component. In this connection, it may be of interest to recall that the main components represent 65%, 69% and 61% of man, calf and mouse total DNAs, respectively, if the reference is the sum of the major components instead of total DNA. All the figures quoted are from Table 2; guinea pig DNA was not taken into account since its 1·704 and 1·709 g/cm^3 components appear to be exceptionally low.

A novel feature of the main component of mammalian DNAs is that it is made up of two sub-components. According to the data of Table 2 these sub-components significantly differ, in different mammalian DNAs, in both buoyant densities (1·696 to 1·699 and 1·699 to 1·7015 g/cm^3, respectively) and relative amounts (16 to 45% and 22 to 49% of total DNAs, respectively). It is difficult to understand at the present time the significance of these sub-components. Two points should be noted, however: (1) that the sub-components vary in buoyant densities and amounts in species which are evolutionarily not far from each other; some mammalian genomes only show a single main component of density 1·699 to 1·700 g/cm^3 (Table 3); (2) that these variations appear to exist in different tissues from the same species (Table 3). It may

be wondered whether these differences are not related to a fractionation of DNA during its preparation or to differences in the breed or sex of animals. Differences in the main components from DNAs prepared in parallel from five different tissues of a single calf foetus (work to be published) seem, however, to argue in favour of a real difference in the main components. In view of the slight differences in buoyant density of the sub-components, and of their species and tissue-dependent variability, it may be interesting to consider the possibility that sub-components of the main components of mammalian DNAs differ in their level of methylation.

The 1·704 and 1·709 g/cm^3 components of mammalian genomes are remarkably similar to each other in their buoyant densities, the maximum differences only reaching 1 mg/cm^3. They are also very similar in their relative amounts: 20 to 25% for the 1·704 g/cm^3 component and about 10% for the 1·709 g/cm^3; as in the case of the main component, this range narrows down if one disregards the minor and satellite components, which vary from one species to another. The higher values reported in Table 3 (39% and 29% for the 1·704 g/cm^3 components of rat and hedgehog, respectively; 15% for the 1·709 g/cm^3 of garden dormouse) may be due to the contribution of cryptic satellites; this explanation, justified by the absence or very low amounts of satellite bands in these genomes, may be checked by preparative Cs_2SO_4–Ag^+ density gradient centrifugation. As already mentioned, the guinea pig genome has an exceptionally low amount of both components.

The similarity of the major components of mammalian DNAs is in sharp contrast with the situation prevailing in some satellite DNAs, which vary considerably in buoyant density even in closely related species (Walker, 1971).

A much greater number, 93, of mammalian DNAs has been analyzed by sedimentation in CsCl by Arrighi *et al.* (1970), but these investigations have been limited to the determination of ρ_0 of main band and apparent satellites. It should also be noted that components similar in buoyant densities and amounts, to those described in this paper were detected by CsCl preparative centrifugation in the DNAs from mouse and rat (McConaughy & McCarthy 1970).

(b) *Other eukaryotic DNAs*

It should be noted that the number of these DNAs examined is very small and that some generalizations given below can only be considered as tentative.

The two avian DNAs present a component pattern, in CsCl, which is remarkably similar to those of mammalian DNAs. It remains, however, to be established whether some kind of homology exists between the 1·704 and 1·709 g/cm^3 components of mammals and birds. If this was shown to be the case, the heavy component found in the DNAs of reptiles, which are the common ancestors of birds and mammals, might be the precursor of those components.

The amphibian DNAs definitely show major components which are very different from those of higher vertebrates and from each other. The latter differences might somehow be related to the large differences in the genome sizes of the two amphibians investigated here.

The two fish DNAs were characterized by a very high degree of symmetry of their CsCl bands. The single DNA component of these vertebrates, characterized by a particularly low *c*-value (about 1 pg DNA/haploid cell), could be the ancestor of the main component of evolutionarily more recent vertebrates.

As far as the three invertebrate DNAs are concerned, the *Drosophila* main band

DNA is again characterized by a very high degree of symmetry, whereas the two sea urchin DNAs show a small degree of asymmetry which should be further studied by Cs_2SO_4–Ag^+ density gradient centrifugation.

Finally, the three unicellular eukaryote DNAs were characterized by CsCl bands which were perfectly symmetrical, in fact more so than that of *E. coli* DNA. It should be noted that the genome size of the unicellular eukaryotes examined covered an extremely wide range (0·025 to 14 pg DNA/haploid cell).

The existence of a number of major, discrete components in the genomes of 11 mammals belonging to five different orders is an important finding, which raises a number of questions. The most important ones concern the evolutionary origin, and the biological significance of the components. As far as the first point is concerned, more work has to be done to check whether the suggestions put forward above are correct. An important conclusion seems, however, to be established by our results, namely, that large-scale changes in the genome organization have taken place during the evolution of vertebrates. The relative arrangement of the components in the mammalian genome and their intramolecular heterogeneity will be discussed in the following paper. The biological significance of the components is under active investigation in our laboratory.

We thank Dr J. Filipski for the Cs_2SO_4–Ag^+ analysis of guinea pig DNA and Dr J. E. Hearst for a discussion of this work.

REFERENCES

Arrighi, F. E., Mandel, M., Bergendahl, J. & Hsu, T. C. (1970). *Biochem. Genet.* **4**, 367–376.
Barsacchi, G. & Gall, J. G. (1972). *J. Cell Biol.* **54**, 580–591.
Becak, W., Shreiber, G., Lavalle, D. & Oliveira-Amorim, F. (1970). *Experientia*, **26**, 204–206.
Bernardi, G., Faurès, M., Piperno, G. & Slonimski, P. (1970). *J. Mol. Biol.* **48**, 23–42.
Birnstiel, M., Speirs, J., Purdom, I., Jones, K. & Loening, V. E. (1968). *Nature (London)*, **219**, 459–463.
Brown, D. D. & Sugimoto, K. (1974). *Cold Spring Harbor Symp. Quant. Biol.* **38**, 501–505.
Corneo, E., Ginelli, E. & Polli, E. (1967). *J. Mol. Biol.* **23**, 619–622.
Corneo, G., Ginelli, E. & Polli, E. (1968*a*). *J. Mol. Biol.* **33**, 331–335.
Corneo, G., Ginelli, E., Soave, C. & Bernardi, G. (1968*b*). *Biochemistry*, **7**, 4373–4379.
Corneo, G., Ginelli, E. & Polli, E. (1970). *J. Mol. Biol.* **48**, 319–327.
Corneo, G., Ginelli, E. & Polli, E. (1971). *Biochim. Biophys. Acta*, **247**, 528–534.
Corneo, G., Zardi, L. & Polli, E. (1972). *Biochim. Biophys. Acta*, **269**, 201–204.
Filipski, J., Thiery, J. P. & Bernardi, G. (1973). *J. Mol. Biol.* **80**, 177–197.
Ifft, J. B., Voet, D. M. & Vinograd, J. (1961). *J. Phys. Chem.* **65**, 1138–1145.
Kay, E. R. M., Simmons, N. S. & Dounce, A. L. (1952). *J. Amer. Chem. Soc.* **74**, 1724–1726.
Macaya, G., Bernardi, G. & Thiery, J.-P. (1976*a*). In *Chromosomes in Biology and Medicine* (Yunis, J. J., ed.), vol. 1, Academic Press, New York. in the press.
Macaya, G., Thiery, J.-P. & Bernardi, G. (1976*b*). *J. Mol. Biol.* **108**, 237–254.
McConaughy, B. L. & McCarthy, B. G. (1970). *Biochem. Genet.* **4**, 425–446.
Meselson, M., Stahl, F. W. & Vinograd, J. (1957). *Proc. Nat. Acad. Sci., U.S.A.* **43**, 582–588.
Prunell, A. & Bernardi, G. (1973). *J. Biol. Chem.* **248**, 3433–3440.
Schildkraut, C. L. & Maio, J. J. (1969). *J. Mol. Biol.* **46**, 305–312.
Schmid, C. W. & Hearst, J. E. (1969). *J. Mol. Biol.* **44**, 143–160.
Schmid, C. W. & Hearst, J. E. (1971). *Biopolymers*, **10**, 1901–1924.
Schmid, C. W. & Hearst, J. E. (1972). *Biopolymers*, **11**, 1913–1918.
Sinclair, J. M. & Brown, D. D. (1971). *Biochemistry*, **10**, 2761–2769.
Stutz, E. & Bernardi, G. (1972). *Biochimie*, **54**, 1013–1021.
Sueoka, N. (1959). *Proc. Nat. Acad. Sci., U.S.A.* **45**, 1480–1490.

Sueoka, N. (1961). *J. Mol. Biol.* **3**, 31–40.
Sueoka, N. (1962). *Proc. Nat. Acad. Sci., U.S.A.* **48**, 582–592.
Szybalski, W. (1968). In *Methods in Enzymology* (Grossman, L. & Moldave, K., eds), vol. 12, part B, pp. 330–360, Academic Press, New York.
Thiery, J. P., Ehrlich, S. D., Devillers-Thiery, A. & Bernardi, G. (1973). *Eur. J. Biochem.* **38**, 434–442.
Walker, P. M. B. (1971). *Prog. Biophys. Mol. Biol.* **23**, 145–190.
Whittaker, R. H. (1969). *Science*, **163**, 150–160.