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An Analysis of the Bovine Genome by Density-Gradient Centrifugation

Preparation of the dG+dC-Rich DNA Components

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The dG+dC-rich fractions obtained by density gradient centrifugation of bovine DNA in Cs_2SO_4/BAMD [J. Cortadas, G. Macaya & G. Bernardi (1977) *Eur. J. Biochem. 76*, 13–19] were centrifuged in Cs_2SO_4/Ag^+ density gradients. These experiments led to the preparation of the DNA components which had been detected (by analytical centrifugation in CsCl) in the Cs_2SO_4/BAMD fractions, and also of DNA components which had identical behaviors in Cs_2SO_4/BAMD gradients and identical buoyant densities in CsCl. A total of eight satellite components and 11 minor components, accounting for 23% and 4% of the bovine genome, respectively, were thus isolated and characterized in their relative amounts and buoyant densities. The implications of these results on the interpretation of renaturation kinetic data on the bovine genome are discussed.

Centrifugation of calf thymus DNA in preparative Cs_2SO_4/BAMD density gradients has led to the separation of dG+dC-rich fractions which have been analyzed in terms of DNA components by analytical centrifugation in CsCl [1]. In the present work, we have prepared these components by submitting the Cs_2SO_4/BAMD fractions to further centrifugations in Cs_2SO_4/Ag^+ density gradients. This has also led to the preparation of DNA components having the same behavior in Cs_2SO_4/BAMD density gradients and the same buoyant density in CsCl. A precise assessment of the buoyant density and relative amount of eight satellite components and 11 minor components so isolated from the bovine genome has thus been possible.

As in other recent papers from this laboratory, (quoted in [1]), we call here DNA components the populations of genome fragments which can be separated from each other by density gradient centrifugation techniques. We distinguish three groups of DNA components according to their nucleotide sequence patterns and relative amounts in the genome as follows. (a) Satellite components are formed by short repeated nucleotide sequences; each satellite component usually represents a small percentage of the genome. (b) Minor components each account for less than 3% of the genome; they are not formed by short repeated nucleotide sequences, yet they may contain a certain amount of them; several, if not all, minor components are formed by repeated genes. (c) The three or four major DNA components, making up the bulk of mammalian genomes, contain interspersed unique and repetitive sequences (unpublished results). The different features of nucleotide sequences in these three classes of DNA components allow a distinction to be made on the basis of a number of physical and chemical properties. Suffice it to mention here that the electrophoretic patterns of fragments produced by different restriction enzymes provide a rapid criterion for such a distinction (see following paper). Satellite components are characterized by banding patterns in which the molecular weights of the fragments reveal the underlying repetitive organization of the nucleotide sequences. Major components show continuous distributions of fragment sizes, in which no bands are detected. Minor components either show simple banding patterns due to the tandem repetition of gene-spacer units or continuous distributions of fragments. A detailed discussion of the properties of different DNA components will be presented elsewhere.

Abbreviations. BAMD, 3,6-bis(acetato-mercurimethyl)dioxane; r_f, molar ratio of ligand to DNA phosphate.

MATERIALS AND METHODS

DNA Preparation

The starting material for the Cs_2SO_4/Ag^+ preparative centrifugation were the fractions from the Cs_2SO_4/

BAMD experiment shown in Fig. 5 of a previous paper [1]; this figure is reproduced here as Fig. 2. Material from 12 tubes centrifuged simultaneously was used, after pooling the corresponding fractions. Each pooled fraction was dialyzed exhaustively, first against 2 M NaCl, to eliminate BAMD, and then against either 0.005 M $Na_2B_4O_7$, pH 8.1 or 9.2, or 0.01 M sodium phosphate, pH 6.8.

Centrifugation Experiments

Preparative centrifugation of DNA/Ag^+ complexes was done as already described [2]. Recentrifugation experiments were done after exhaustive dialysis against 2 M NaCl and the appropriate buffer.

Analytical centrifugation in CsCl density gradient was as previously described [1].

RESULTS

Conditions for the Optimal Resolution of DNA Components in Cs_2SO_4/Ag^+ Density Gradients

These conditions were investigated by studying the effect of pH and r_f in analytical centrifugation experiments which were carried out on each Cs_2SO_4/BAMD fraction from the preparative density gradient of Fig. 2 (see next section). Such experiments have led to a better understanding of the effect of these parameters on DNA fractionation in Cs_2SO_4/Ag^+ density gradients.

It is known that the affinity of Ag^+ is higher for dG + dC-rich DNAs at pH $\leq$ 7, whereas it is higher for dA + dT-rich DNAs at pH $\geq$ 8 [2]. This is due to the existence of two types of binding sites, the first one involving dG · dC base pairs, the second one dA · dT base pairs; Ag^+ binding on the first type of sites does not cause an increase in buoyant density, whereas Ag^+ binding on the second type of sites does [3–5]. Furthermore, at pH $\leq$ 7, Ag^+ binding is linearly related to the base composition of DNA [3], whereas at pH $\geq$ 8 sequence effects on the binding are evident [2] (and below). It should also be added that the overall affinity of Ag^+ for DNA is lower at pH $\leq$ 7 [4, 5]; this explains why under these conditions the effect of r_f on buoyant density is lower and the choice of the initial density of Cs_2SO_4 is less critical.

Fig. 1 shows an example of the effect of r_f on the resolution of DNA components. In this case (corresponding to fraction 7 from the Cs_2SO_4/BAMD gradient, see Fig. 2 and 9), separation of components is improved by increasing the r_f value above 0.1; an optimal separation is obtained at an r_f value around 0.3, whereas at higher r_f values (for instance, at r_f = 0.4) the two intermediate components are no longer resolved (Fig. 1 A); for this reason r_f values higher than 0.4 were not examined.

The plot in Fig. 1 B deserves three comments. (a) The addition of silver below a certain r_f threshold does not cause an increase in buoyant density; this phenomenon, particularly evident for component d of Fig. 1 A, may be due to the formation of an Ag^+ · DNA complex of the first type. (b) The lightest component (component a of Fig. 1 A) does not increase its density under the conditions used; this may be due either to a lack of binding of Ag^+ to this component, or, more likely, to the fact that the Ag^+ · DNA complex formed is a complex of the first type. (c) The increase in buoyant density with r_f is different for different components because of their different binding constants for Ag^+.

All the above data imply that the choice of the initial density of the Cs_2SO_4 solution should be done on the basis of the r_f value used, and of the particular DNA components present in the mixture under consideration. In this work, the initial density of the Cs_2SO_4 solution was chosen so as to have the center of gravity of the DNA components close to the center of the density gradient.

Fractionation of DNA Components in Cs_2SO_4/Ag^+ Density Gradients

Fig. 2 shows the preparative Cs_2SO_4/BAMD density gradient centrifugation experiment (already commented on in [1]) which yielded the fractions submitted to further fractionation in Cs_2SO_4/Ag^+ density gradients.

Fig. 3–10 present the Cs_2SO_4/Ag^+ density gradient centrifugations leading to the preparation of DNA components from each Cs_2SO_4/BAMD fraction. In each case optimal conditions were chosen on the basis of the criteria given above. Each fraction from the Cs_2SO_4/Ag^+ preparative experiments was analyzed by analytical CsCl density gradient centrifugation and the density profiles so obtained are shown. DNA components appearing in these profiles were therefore characterized by their behavior in Cs_2SO_4/Ag^+ and by their buoyant density in CsCl (the values are given in the figures). These properties were usually sufficient to establish the identity of DNA components as derived from neighboring fractions. Sometimes, however, components having a different behavior in Cs_2SO_4/Ag^+ showed the same buoyant density in CsCl. In such case, as well in every case of doubt, the criterion used for establishing the identity of components was the electrophoretic pattern shown upon degradation with several restriction enzymes (see the following paper [6]).

To illustrate the procedure just described we will discuss here Fig. 9 as an example. In this case, fraction 7 from the Cs_2SO_4/BAMD density gradient (Fig. 2) showed three peaks and a shoulder when centrifuged in Cs_2SO_4/Ag^+ under optimal r_f and pH (see Fig. 1;

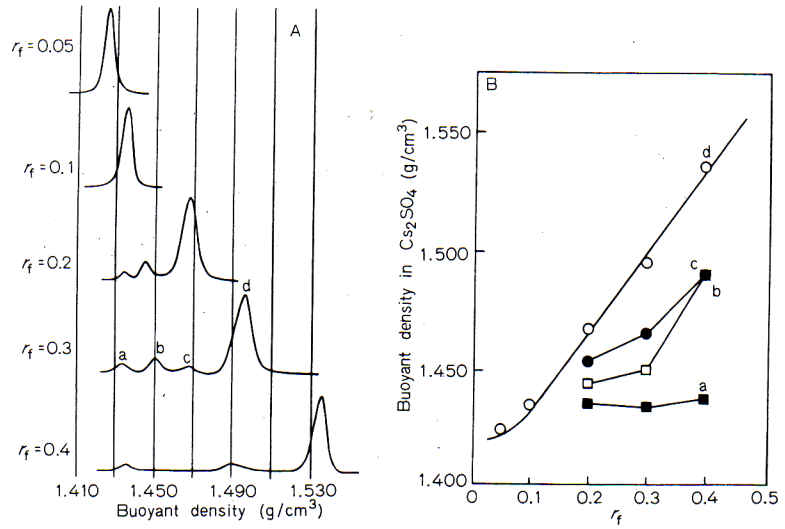

Fig. 1. *Effect of* r_f *on the resolution of DNA components.* Aliquots (0.05 A_{260} unit) of fraction 7 (Fig. 2) in 5 mM $Na_2B_4O_7$, pH 9.2, were centrifuged in a model E Spinco analytical ultracentrifuge at 44000 rev./min and 25 °C in the presence of Ag^+, at increasing values of r_f. (A) Analytical profiles at different r_f values. (B) Buoyant density of the components, a, b, c, and d, plotted as a function of r_f

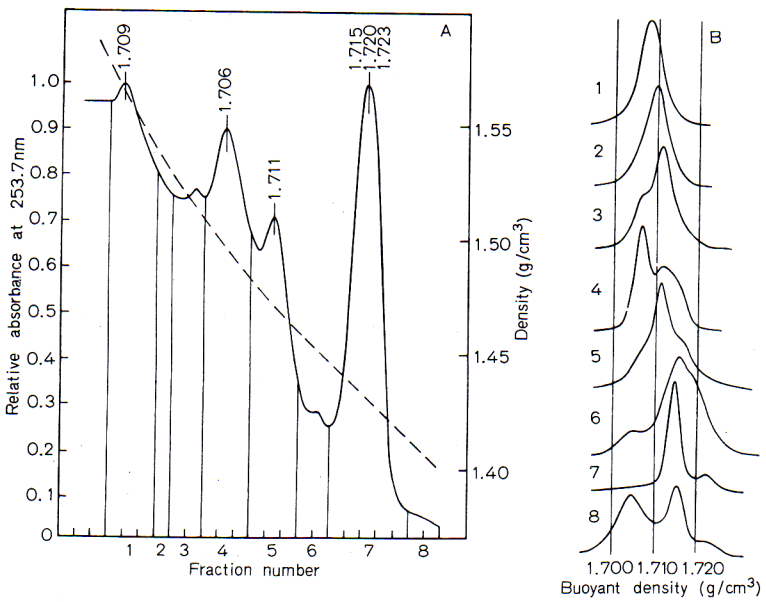

Fig. 2. *Fractionation of calf thymus DNA in* Cs_2SO_4*/BAMD density gradient at* r_f = *0.18 [1].* Calf thymus DNA (210 A_{260} units) in 0.1 M Na_2SO_4, 5 mM $Na_2B_4O_7$, pH 9.2, containing BAMD (r_f = 0.18) and Cs_2SO_4 (ϱ = 1.46 g/cm³), was centrifuged in a Beckman type 30 rotor at 25 °C for 110 h at 25000 rev./min. (A) The relative absorbance profile of the centrifuged DNA solution as derived from the transmission recordings at 253.7 nm. The buoyant densities in CsCl of the satellite components present in each peak are indicated. The broken line indicates the density gradient. (B) The analytical band profiles in CsCl of each fraction

the better resolution in the analytical gradient compared to the preparative one is essentially due to a difference in the slope of the gradient). The Cs_2SO_4/Ag^+ density gradient was split into four fractions, 71–74. CsCl analytical centrifugation of these fractions showed the presence of four components banding in CsCl at 1.706, 1.715, 1.720 and 1.723 g/cm³. The three latter components had already been detected analytically in previous work (see Fig. 3–5 of [1]); the fourth component (1.706 g/cm³) was present in very small amount; for this reason it was not seen in the CsCl profile of fraction 7 [1] (and Fig. 2).

Fraction 71 was shown to be composed of pure 1.715-g/cm³ satellite by its CsCl profile (Fig. 9) and restriction pattern (Fig. 3 of [6]).

Fraction 72 was formed by a peak and a shoulder; CsCl centrifugation of this fraction indicated that the peak corresponded to the 1.723-g/cm³ satellite and the shoulder to a mixture of contaminating 1.715-g/cm³ satellite plus a new DNA component; since the overall

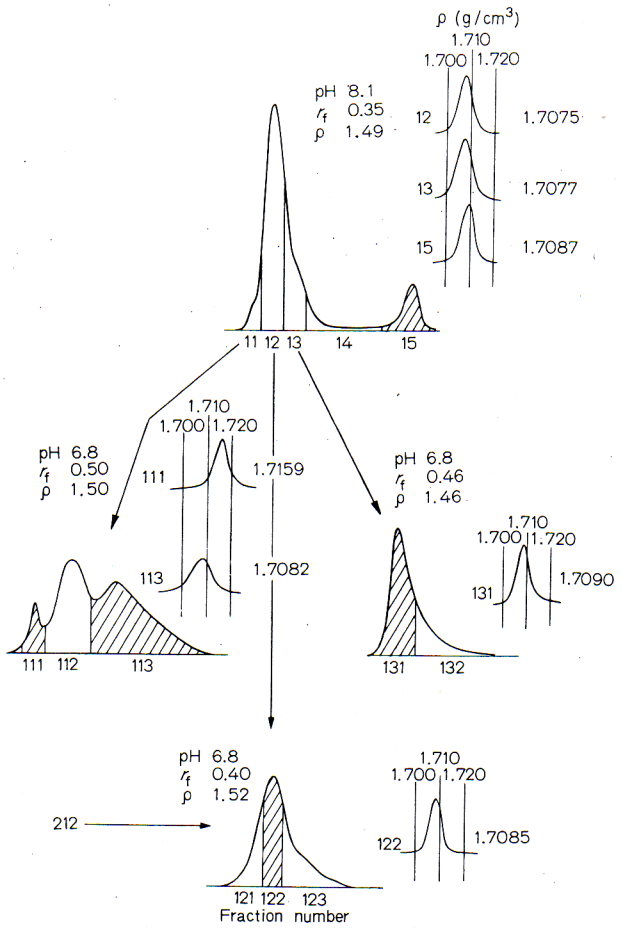

Fig. 3. *Preparative Cs_2SO_4/Ag^+ centrifugation of the Cs_2SO_4/BAMD fractions (Fig. 2) of calf thymus DNA: fraction 1.* Fractions are numbered according to the sequential steps of purification in Cs_2SO_4/Ag^+ density gradients: for instance, fractions 111, 112 and 113 are derived from fraction 11, which is in turn derived from fraction 1 of the original Cs_2SO_4/BAMD density gradient (Fig. 2). The Cs_2SO_4/Ag^+ profiles are absorbance profiles at 253.7 nm derived from the ultraviolet transmission recordings; density increases from right to left. The fractionation conditions (pH, r_f, and initial gradient density) are indicated. Hatched fractions represent purified components on the basis of the analytical CsCl profiles shown in the figure, and on the basis of restriction patterns [6]. The peak corresponding to fractions 112 is an artefact, as shown by the absence of any material banding in CsCl

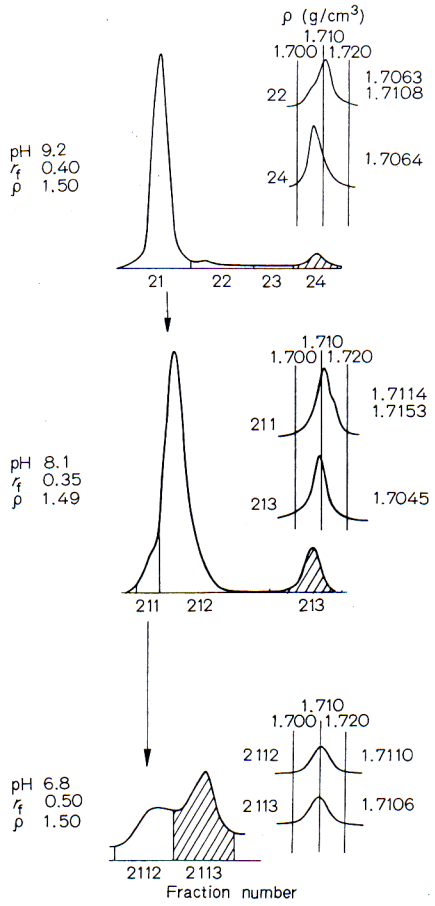

Fig. 4. *Preparative Cs_2SO_4/Ag^+ centrifugation of the Cs_2SO_4/BAMD fractions (Fig. 2) of calf thymus DNA: fraction 2.* Details as in Fig. 3

density was found to be equal to 1.7163 g/cm³, clearly the new component had a buoyant density higher than this value. In order to separate these components, fraction 72 was submitted to another centrifugation in Cs_2SO_4/Ag^+. Analytical centrifugation in CsCl of the resulting fractions revealed that fraction 721 contained essentially pure 1.715-g/cm³ satellite (Fig. 3 of [6]), fraction 722 was very highly enriched in a 1.720-g/cm³ component and fractions 723 and 724 were essentially formed by 1.723-g/cm³ satellite, the latter fraction also containing a very small amount of 1.706-g/cm³ component. Upon another Cs_2SO_4/Ag^+ centrifugation, fraction 723 yielded a fraction (7232) formed by the 1.723-g/cm³ satellite, whereas fraction 722 yielded a fraction, (7222), formed by the 1.720 g/cm³ component (Fig. 4 of [6]) plus two fractions (7221 and 7223) in which this component was contaminated by the 1.715-g/cm³ and 1.723-g/cm³ satellites, respectively.

Fraction 74 was also submitted to a further Cs_2SO_4/Ag^+ density gradient centrifugation. This yielded a 1.720-g/cm³ component (fraction 743) as well as two fractions (741 and 742) mainly formed by the 1.715-g/cm³ and 1.706-g/cm³ satellites, respectively. The 1.720-g/cm³ component of fraction 743 differed from the 1.720-g/cm³ component of fraction 7222 in its behavior in Cs_2SO_4/Ag^+ density gradient (Fig. 9) and in its restriction pattern [6]; these two satellites will be indicated here as 1.720a (7222) and 1.720b (743) respectively. In contrast, the 1.715-g/cm³ component of fraction 741 and the 1.706-g/cm³ component of fraction 742 were found by restriction analysis to be identical with the 1.715-g/cm³ satellite of fractions 71 and 721 and 612 (Fig. 3 of [6]) and with the 1.706-g/cm³ satellite of fraction 48, respectively.

The relative amount of DNA components in each fraction was determined by measuring the areas under the peaks of the preparative densitograms shown in

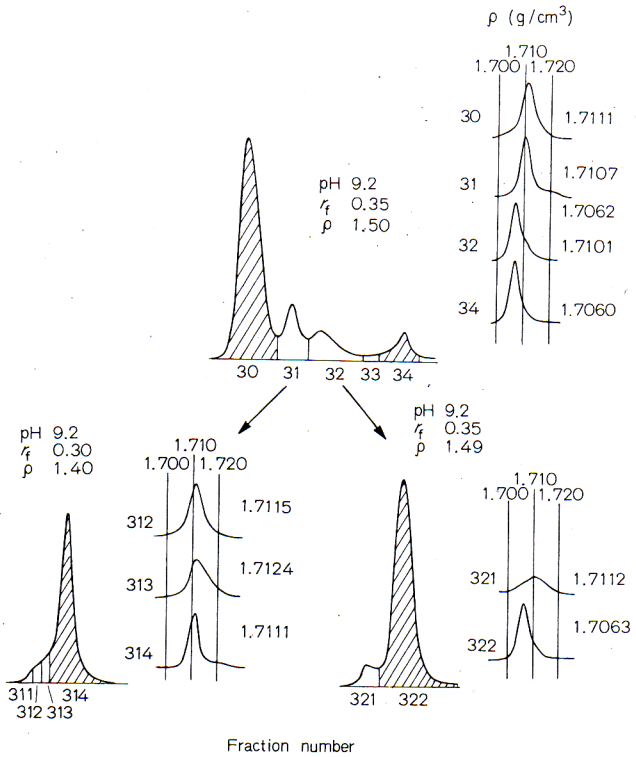

Fig. 5. *Preparative Cs_2SO_4/Ag^+ centrifugation of the Cs_2SO_4/BAMD fractions (Fig. 2) of calf thymus DNA: fraction 3.* Details as in Fig. 3

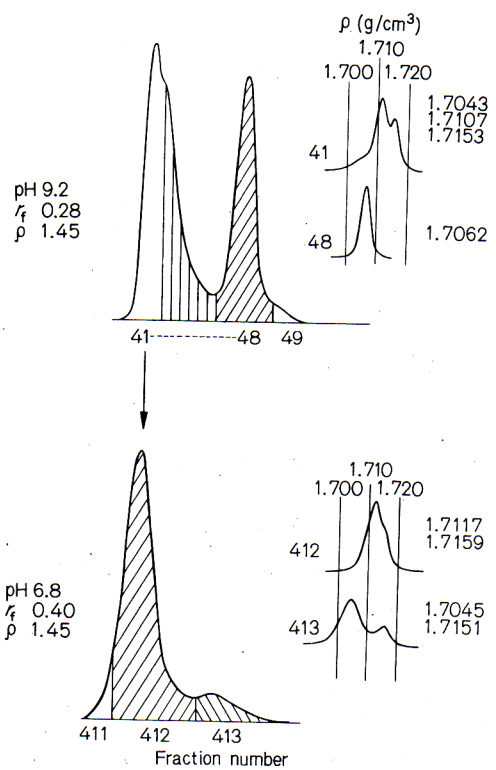

Fig. 6. *Preparative Cs_2SO_4/Ag^+ centrifugation of the Cs_2SO_4/BAMD fractions (Fig. 2) of calf thymus DNA: fraction 4.* Details as in Fig. 3

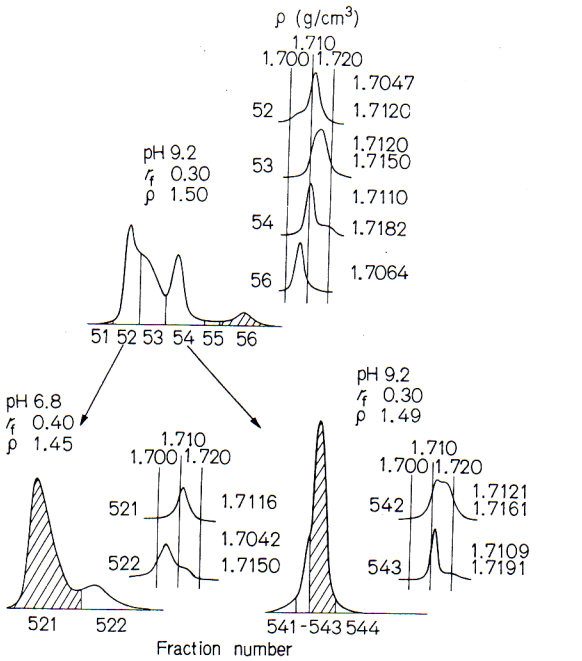

Fig. 7. *Preparative Cs_2SO_4/Ag^+ centrifugation of the Cs_2SO_4/BAMD fractions (Fig. 2) of calf thymus DNA: fraction 5.* Details as in Fig. 3

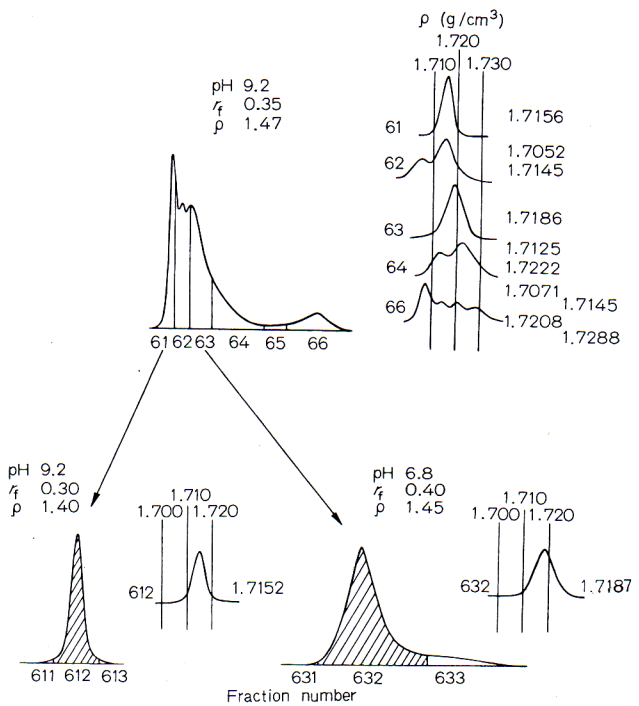

Fig. 8. *Preparative Cs_2SO_4/Ag^+ centrifugation of the Cs_2SO_4/BAMD fractions (Fig. 2) of calf thymus DNA: fraction 6.* Details as in Fig. 3

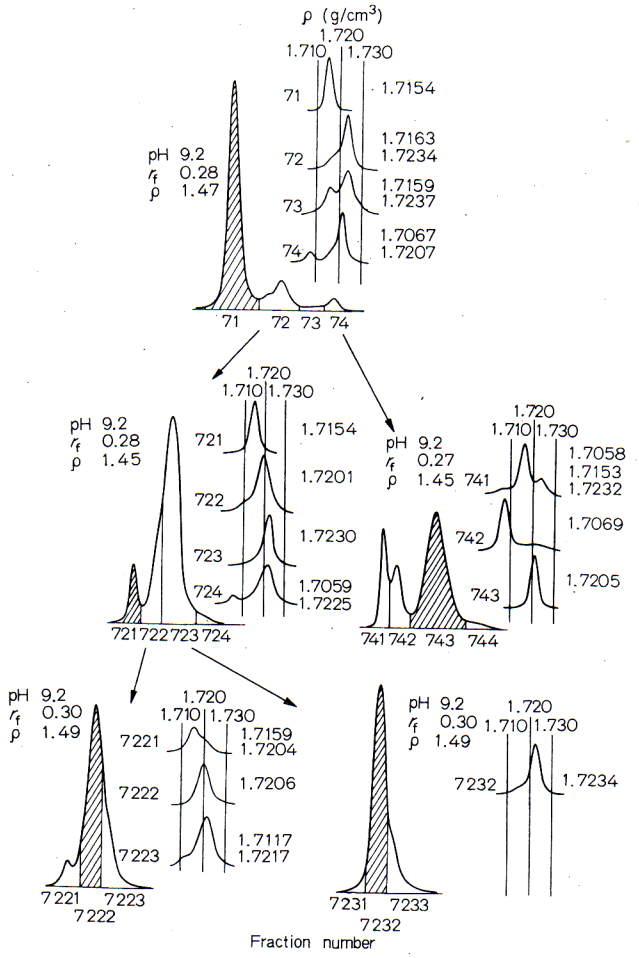

Fig. 9. *Preparative Cs_2SO_4/Ag^+ centrifugation of the Cs_2SO_4/BAMD fractions (Fig. 2) of calf thymus DNA: fraction 7.* Details as in Fig. 3

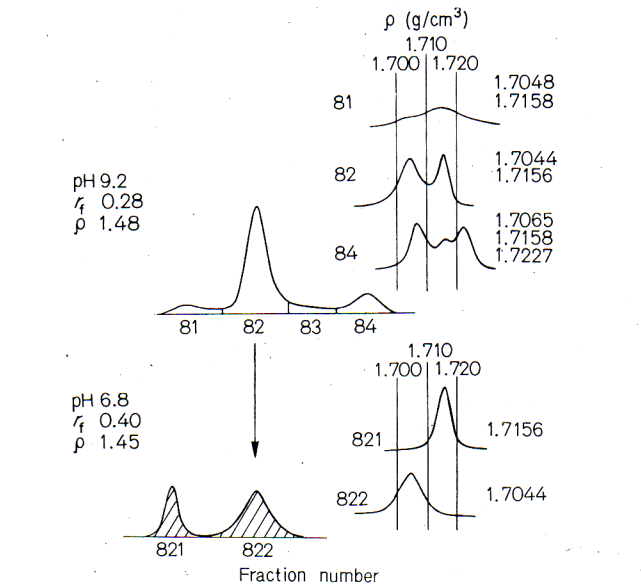

Fig. 10. *Preparative Cs_2SO_4/Ag^+ centrifugation of the Cs_2SO_4/BAMD fractions (Fig. 2) of calf thymus DNA: fraction 8.* Details as in Fig. 3

Table 1. *Calf thymus DNA components*
The criteria used to construct this table are given in the text. Values in bold type correspond to the buoyant density and the relative amount of each component in calf thymus DNA. The fractions labelled (*r*) were tested by restriction enzymes [6]. Letters a–d following the fraction numbers indicate the different components (lightest to heaviest) present in the fractions showing a multimodal CsCl profile

Fraction	ϱ	Amount
	g/cm³	%
Major components		
122	1.7085	4.63 (*r*)
113	1.7082	0.56
2113	1.7090	0.26
	1.7085	**5.45**
Minor components		
413a	1.7045	0.18
522a	1.7042	0.22
	1.7043	**0.40**
822	**1.7044**	**0.12**
62a	1.7052	0.11
724a	1.7059	0.02
	1.7055	**0.13**
24	**1.7064**	**0.31** (*r*)
2112	**1.7110**	**0.18**
7223a	**1.7117**	**0.003**
53a	1.712	0.81
64a	1.7125	0.03
	1.712	**0.84**
412b	1.7159	0.22
413b	1.7151	0.09
522b	1.715	0.09
53b	1.715	0.81
542b	1.7161	0.09
	1.715	**1.30**
111	**1.7159**	**0.09**
632	**1.719**	**0.34** (*r*)
66d	**1.7288**	**0.02**
Satellite components		
322	1.7063	0.46 (*r*)
34	1.7060	0.28 (*r*)
48	1.7062	3.17 (*r*)
56	1.7064	0.22 (*r*)
66a	1.7071	0.05
741a	1.7058	0.002
742	1.7069	0.02 (*r*)
84a	1.7065	0.02
	1.706	**4.22**
131	1.7090	2.46 (r)
15	1.7087	1.58 (r)
213	1.7095	0.53 (r)
	1.709	**4.57**
314	1.7111	0.51 (r)
542a	1.7111	0.10
543	1.7114	1.06 (r)
	1.711a	**1.67**
30	1.7111	3.26 (r)
412a	1.7117	2.28 (r)
521	1.7116	1.54 (r)
	1.711b	**7.08**
612	1.7152	0.22 (r)
62b	1.7145	0.22
71	1.7154	4.32 (r)

Table 1 (Continued)

Fraction	ϱ	Amount
	g/cm³	%
Satellite components		
721	1.7154	0.05 (r)
7221a	1.7159	0.01
7232a	1.7177	0.04
741b	1.7153	0.02 (r)
821	1.7156	0.18
84b	1.7158	0.013
	1.715	**5.07**
7221b	1.7204	0.004
7222	1.7206	0.14 (r)
	1.720a	**0.14**
743	**1.720b**	**0.11** (r)
64b	1.723	0.06
7223b	1.7217	0.04
7232b	1.7234	0.33 (r)
724b	1.723	0.05
741c	1.7232	0.007
84c	1.7227	0.02
	1.723	**0.51**

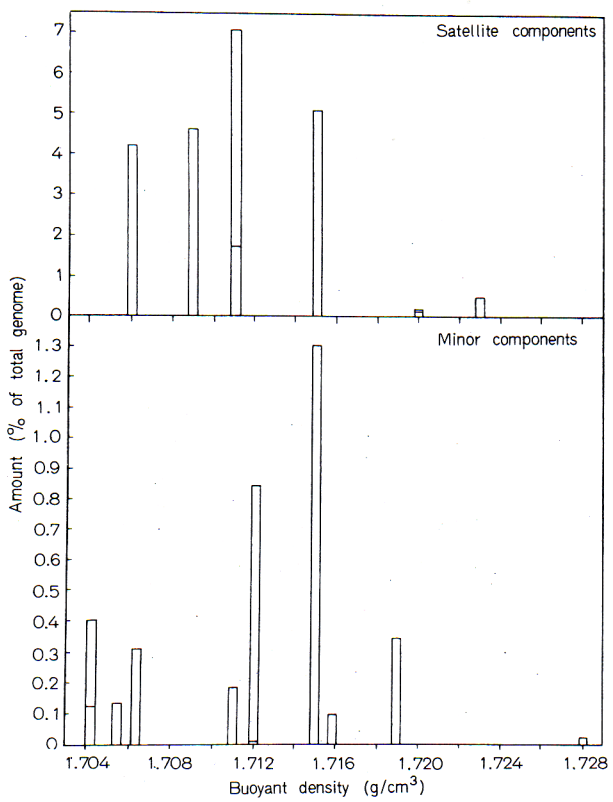

Fig. 11. *Histogram of satellite and minor components of calf thymus DNA as derived from Table 2.* Horizontal lines on some bars indicate the level of a second component having the same buoyant density in CsCl

Fig. 3–10. In most cases, a given fraction only contained a single DNA component; when the main component present in a fraction was accompanied by other components, as judged by the CsCl profile, the relative amounts of the latter were assessed on the basis of a gaussian curve analysis of the analytical CsCl profiles [7].

This kind of analysis allowed us to assemble in Table 1 the fractions corresponding to the DNA components, and to assess their relative amounts. All values were corrected by taking into account the fact that the material present in the preparative Cs_2SO_4/BAMD gradient represented only 40% of the genome [1]. Fig. 11 present in a histogram form the data concerning the buoyant densitities and relative amounts of the satellite and minor components of the bovine genome.

As indicated by Table 1 and Fig. 11, under the conditions used in the Cs_2SO_4/BAMD fractionation, only the heaviest major component (1.7085 g/cm³) of the calf genome was obtained, the three lighter major components being pelleted to the bottom of the centrifuge tube. Of the minor components, only one, namely the 1.715-g/cm³ component, represented more than 1% (1.30%) of the genome; another relatively abundant minor components was the 1.712-g/cm³ component (0.84%); of the remaining minor components, six were in the 0.1–0.4% range and three were below the 0.1% mark. Among the satellite components, four were in the 4–7% range, and four in the 0.1–1.7% range.

DISCUSSION

Table 2 summarizes the results obtained in the present work and compares them with those previously reported [1]. Several differences exist between the two sets of data; these will be discussed here.

Minor Components

The increased number of minor components found here compared to previous work, 11 versus 5, is due to the fact that the larger amount of material processed increases the sensitivity of detection. Concerning the significance of minor components, so far, we have evidence from hybridization experiments that the 1.719-g/cm³ minor component contains ribosomal cistrons (M. Meunier, personal communication), in agreement with an independent report by other authors [8]. It is possible that other minor components may well correspond to other repetitive genes.

A minor component representing as little as 0.003% of total DNA has been detected; since the DNA content per bovine haploid cell is 4.2 pg [9], this component only represents about 80×10^6 daltons of DNA, a value numerically not much higher than the molecular weight (13×10^6) of the DNA preparation used here. This indicates that, under very favorable conditions, a large DNA fragment present in a

Table 2. *Calf thymus DNA components*

Cortadas *et al.* [1]		This work	
ϱ	Amount	ϱ	Amount
g/cm³	%	g/cm³	%
Major components			
1.7085	9.2	1.7085	5.45
Minor components			
	4.5		3.73
		1.7043	0.40
		1.7044	0.12
		1.7055	0.13
1.7065	0.4	1.7064	0.31
1.7110	0.8	1.711	0.18
		1.7117	0.003
		1.712	0.84
1.7150	2.2	1.715	1.30
		1.7159	0.09
1.7190	1.0	1.719	0.34
1.7280	0.1	1.728	0.02
Satellite components			
	18.2		23.37
1.706	4.1	1.706	4.22
1.709	–	1.709	4.57
1.711	3.3	1.711a	1.67
1.7115	4.3	1.711b	7.08
1.715	5.4	1.715	5.07
1.720	0.5	1.720a	0.14
		1.720b	0.11
1.723	0.6	1.723	0.51

single copy in the genome can be isolated by density gradient centrifugation.

Satellite Components

A satellite component having a buoyant density of 1.709 g/cm³ has been recognized in this work by fractionating in Cs_2SO_4/Ag^+ fractions 1 and 2 from Cs_2SO_4/BAMD. The satellite was found in three sub-fractions (fractions 131 and 15, Fig. 3; fraction 213, Fig. 4), but the restriction enzyme patterns of these sub-fractions are identical [6]. Very interestingly, fraction 1 from Cs_2SO_4/BAMD also contains a major component having the density of 1.709 g/cm³; this is found in a Cs_2SO_4/Ag^+ sub-fraction 122 (Fig. 3) and can be readily distinguished from the satellite of identical density because upon restriction enzyme degradation yields a smear instead of a band pattern [6]. The discovery of the 1.709-g/cm³ satellite contaminating the 1.709-g/cm³ major component explains the multimodality of the melting transition and of the CsCl profile (after heat-denaturation and reannealing) of the latter as previously prepared [2]. It also explains why the present estimate of the 1.709-g/cm³ major component is now so much lower, 5.5%, than previously, 9.2% [1]. It should be noticed, however, that because of the very high amount of satellite DNAs in the calf genome, the 1.709-g/cm³ major component still represents 7.5% of major DNA components, a value not too far from those found in other mammals [10].

Two satellites having the same buoyant density in CsCl, 1.711 g/cm³, have been isolated because of their different behavior in Cs_2SO_4/Ag^+; they had already been fractionated in Cs_2SO_4/BAMD (fractions 3, 4 and 5). The restriction enzyme analysis [6] allowed us to confirm that they are different components and to identify the fractions where they are present. Thus, a precise assessment of their relative amounts in the genome has been possible. The first such satellite indicated as 1.711a, has been found in fractions 314, 542a and 543, the second one, indicated as 1.711b, in fractions 30, 412a, and 521 (see Table 1 and Fig. 5–7).

The previously recognized 1.720-g/cm³ satellite [1] has been fractionated into two satellite components (fractions 7222 and 743, Fig. 9); these have the same buoyant density in CsCl, but exhibit a different behavior in Cs_2SO_4/Ag^+ gradients. The non-identity of the two satellites was further confirmed by the restriction enzyme analysis [6]. It should be noted that the estimation of components present in small amounts using the gaussian analysis [1] is subject to gross error when these components are not separated from the main peak; such is the case for the 1.720-g/cm³ satellite component; in contrast, when the estimate is made by gaussian analysis of peaks completely separated from a multimodal CsCl profile the error is lower, as for the 1.723-g/cm³ satellite component.

General Conclusions

Owing to the use of improved density gradient techniques, the number of isolated bovine satellites is now eight, the highest reported so far for an eukaryotic genome. CsCl density gradients revealed the 1.715-g/cm³ [11] and 1.723-g/cm³ [12] satellites, Cs_2SO_2/Ag^+ the 1.706-g/cm³ and 1.711-g/cm³ satellites [2, 13], Cs_2SO_4/BAMD a 1.711-g/cm³ and a 1.720-g/cm³ satellite [1] and the combination of Cs_2SO_4/BAMD with Cs_2SO_4/Ag^+ (present work) the 1.709-g/cm³, a new 1.711-g/cm³ and a new 1.720-g/cm³ satellite (some of the quoted densities are corrected to the values determined in recent work done in our laboratory). The discovery and quantitative estimation of the 1.709-g/cm³ satellite, and the better assessment of the amounts of the satellite components having a buoyant density of 1.711 g/cm³ obtained in the present work, raise the total amount of satellite DNA in the calf genome to a value of 23%, or 1 pg per haploid cell (see above), one of the highest reported so far for mammals. This finding has some general implications (see below).

Table 3. *Components of the bovine genome*
The renaturation kinetics data are taken from [14]; density gradient centrifugation data are from the present work

Method of detection	Type of component	Number	Amount
Renaturation kinetics	non-repetitive	1	55
	intermediate	1	38
	fast	1	2
	very fast	1	3
		4	
Density gradient centrifugation	major	4	73
	minor	11	4
	satellite	8	23
		23	

Table 3 presents a comparison of the analysis of the bovine genome as obtained by renaturation kinetics [14] and by density gradient centrifugation.

Before discussing this comparison, it should be pointed out that the two sets of data differ from one another in three respects. (a) The definition of a DNA component, as identified by density gradient centrifugation, is quite precise, whereas that of a class of DNA sequences sharing common renaturation properties is relatively vague and subjective. (b) The assessment of the amount of a DNA component is again quite precise, whereas that of different renaturing sequence classes is not so, for a number of reasons, among which are their fuzzy limits. (c) The resolving power of density gradient centrifugation is very high, as witnessed by the resolution of 23 DNA components from the bovine genome, as opposed to the four different classes of renaturing sequences seen by the kinetic approach. These drawbacks of renaturation kinetics are essentially due to the great complexity of eukaryotic genomes and, more particularly, to the presence of different sorts of repetitive sequences in them. As expected, a considerable improvement of the renaturation kinetic analysis is obtained when different DNA components are examined after isolation by density gradient centrifugation (unpublished results).

In the case of Table 3, the purpose of the comparison is to see whether any kind of correspondence can be found between the two sets of data and, more specifically, whether an explanation can be found for the fact that the intermediate renaturing class of DNA sequences, having a reiteration frequency of 60000, is so abundant (38%) in the bovine genome, compared to other mammalian genomes.

Disregarding for the present purpose the rapidly renaturing material (3%), which corresponds to DNA sequences folding back upon themselves according to first-order kinetics, it is clear that the fast-renaturing sequences (2%) only represent a minor part of the eight satellite components accounting for 23% of the genome. This leads to the obvious conclusion that most of the satellite sequences appear to renature in the intermediate class; this may be due to sequence divergence within each satellite and/or to cross-hybridization of different satellites or of satellites and interspersed repetitive sequences. The conclusion just drawn means that only about 17% of the material from the intermediate class could correspond to interspersed repetitive sequences. Interestingly enough, a look at the actual renaturation data [15] indicates that the intermediate class of bovine DNA sequences renatures over as much as three decades of c_0t (the product of initial DNA concentration by renaturation time), a clear sign of renaturation heterogeneity. It should be pointed out that the amount of interspersed repetitive sequences, as estimated above (17%), accounts for the difference between the amount of major components seen by density gradient centrifugation (73%) and the amount of non-repetitive sequences (55%) detected by the kinetic method; this suggests that the interspersed repetitive sequences are all present in the major components. Renaturation kinetics of isolated major components of mammalian genomes indicate not only that interspersed repetitive sequences are present in them, but also that the above estimate, 17%, is in the right range.

The above reasoning may mean that a correspondence between renaturing sequence classes and DNA components can be determined. Since such correspondence, at present, can be regarded at best as semi-quantitative, we have not taken into account the facts that (a) the minor components (4%) correspond to the intermediate and/or non-repetitive class, (b) fold-back DNA (2%) can originate from any component and (c) 2% of DNA is unaccounted for in the renaturation data of Table 3.

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