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The DNA Components of the Chicken Genome

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The organization of the chicken genome was investigated by centrifuging chicken DNA ($M_r = 57 \times 10^6$) in preparative Cs_2SO_4/Ag^+ and Cs_2SO_4/BAMD density gradients [BAMD = 3,6-bis(acetato-mercurimethyl)dioxane]. An analysis by CsCl density gradient of the DNA fractions obtained from the preparative experiments revealed that 88% of the genome is made up of four DNA components, characterized by buoyant densities of 1.699, 1.702_5, 1.704_5 and 1.708 g/cm³, and representing 39%, 25%, 15%, and 9%, respectively, of the total DNA. The remaining 12% of the genome is formed by seven minor and/or satellite components. The distribution of the ovalbumin gene in a Cs_2CO_4/BAMD density gradient, as tested with a cloned cDNA probe, coincides with the distribution of the 1.702_5-g/cm³ component. This shows that the DNA regions flanking the ovalbumin gene are homogeneous in base composition over long distances and that the gene is located on a DNA segment belonging to the 1.702_5-g/cm³ component.

Several years ago we showed that the bulk of the bovine genome (namely all the DNA except for satellite and minor components) is not formed by DNA fragments having a continuous distribution of their average dG + dC content, but by three distinct families of fragments [1]. These were characterized by buoyant densities in CsCl equal to 1.697, 1.704 and 1.709 g/cm³, and represented about 50%, 25% and 10% of the total DNA, respectively. When isolated by centrifugation in Cs_2SO_4/Ag^+, these 'major' components of the bovine genome, showed symmetrical CsCl bands, which exhibited standard deviations close to those of bacterial DNAs of comparable molecular weight.

Further work indicated that the three major DNA components, first seen in the bovine genome, were also present in the other ten mammalian DNAs investigated [2, 3]; in addition, it was seen that in several DNAs the lightest and most abundant major component could be resolved into two sub-components. Two avian genomes, when analyzed by CsCl density gradient centrifugation, also appeared to contain similar DNA components. It should be noted that, in mammalian and avian genomes, the major light components are in the same buoyant density range as the DNAs of lower vertebrates, whereas the major heavy components are absent in the DNAs of lower vertebrates.

The major components of several mammalian genomes were characterized by a detailed analysis involving both preparative and analytical ultracentrifugation in density gradients. In contrast, those of the avian genomes were only studied by a gaussian analysis of analytical CsCl profiles of two genomes (chicken and sea gull). As already pointed out [2], such an approach is not as satisfactory as the preceding one and a more precise study was therefore needed in the latter case.

In the present investigation we have analyzed the chicken genome using two density gradient centrifugation techniques, involving Cs_2SO_4/Ag^+ [4] and Cs_2SO_4/BAMD [5]. The results that we have obtained confirm the preliminary observations made by studying analytical CsCl profiles [2], namely that the chicken genome contains, beside several minor and/or satellite components, four major DNA components, having buoyant densities of 1.699, 1.702_5, 1.704_5, and 1.708 g/cm³ and representing 39%, 25%, 15% and 9%, respectively, of the genome.

The distribution of DNA fragments carrying the ovalbumin gene was investigated to obtain information on the compositional homogeneity of the environment of this gene, and on the DNA component containing it. The results obtained indicate that the environment of the ovalbumin gene is homogeneous in base composition over long distances and that the gene is located on a fragment belonging to the 1.702_5-g/cm³ component.

Abbreviations. BAMD, 3,6-bis(acetato-mercurimethyl)dioxane; R_F, molar ratio of ligand (Ag^+ or BAMD) to DNA phosphate.

Enzyme. Restriction endonucleases *Hind*III and *Eco*RI (EC 3.1.4.–) (nomenclature according to Smith and Nathans [17].

MATERIALS AND METHODS

DNA Preparation

DNA was prepared from the erythrocytes of a male white Leghorn chicken. Erythrocytes were lysed in 0.075 M NaCl, 0.024 M EDTA, pH 8.0 [6] containing 0.2% saponin; nuclei were pelleted and washed and the DNA extracted and purified according to Kay et al. [7]. The DNA preparation was further purified by hydroxyapatite chromatography in the presence of 3 M KCl [1]. The sedimentation coefficient s measured by band centrifugation in a Spinco model E analytical ultracentrifuge was found to be equal to 47 ± 1.6 S. This value corresponds to a molecular weight of 56.8×10^6, if the relationship of Eigner and Doty between s and M_r is used.

DNA Fractionation

The DNA-Ag^+ complex was made by adding 1 mM $AgNO_3$ to a concentrated DNA solution in 5 mM $Na_2B_4O_7$, pH 9.2. Cs_2SO_4 (Suprapur, Merck, Darmstadt, F.R.G.) in the same buffer was added to reach a final density of 1.50 g/cm^3. This procedure avoids the precipitation of high-molecular-weight DNA which is caused by the addition of solid Cs_2SO_4.

The DNA-BAMD complex was made as described above, except that the buffer was 5 mM $Na_2B_4O_7$, 0.1 M Na_2SO_4, pH 9.2, and the final density was 1.47 g/cm^3.

Preparative centrifugation and analytical CsCl density gradient centrifugation were done as previously described [5]. The absorbance of the preparative gradients was monitored at 253.7 nm with an LKB Uvicord (Stockholm, Bromma, Sweden) equipped with a cell of 0.3-cm diameter. In order to have a more precise assessment of the amount of DNA in the gradient, the A_{260} of the collected fractions was measured.

A gaussian analysis of the CsCl density gradient profiles of individual or pooled fractions was done using a Dupont de Nemours (Wilmington, Del., U.S.A.) model 310 curve resolver, as previously described [3]. The physical basis of the mathematical curve-fitting used in the present work has been given in detail elsewhere (Appendix 1 of [3]).

Hybridization Experiments

Aliquots of the different Cs_2SO_4/BAMD pools (pellet, fractions 2–6, 7–12, 13–17 and 18–23) containing 13 µg DNA each were digested either by *Hin*dIII or *Eco*RI restriction endonucleases and run on 0.8% agarose gels. DNA digestion with restriction endonucleases, gel electrophoresis, molecular weight standards, transfer of denatured DNA restriction fragments from the gel onto nitrocellulose filters and autoradiography were as already described [8].

The ^{32}P-labelled ovalbumin gene probe, fragment *Hha*I-A of clone pCRI ov2.1, (*Hha* ov), was a gift of Dr P. Chambon. Hybridization of the probe onto chicken DNA fragments was done as described [9].

RESULTS

Fig. 1 A shows the absorbance profile of chicken DNA as centrifuged in a preparative Cs_2SO_4/Ag^+ density gradient at an R_F of 0.40. The DNA was distributed in a narrow peak trailing on the light side

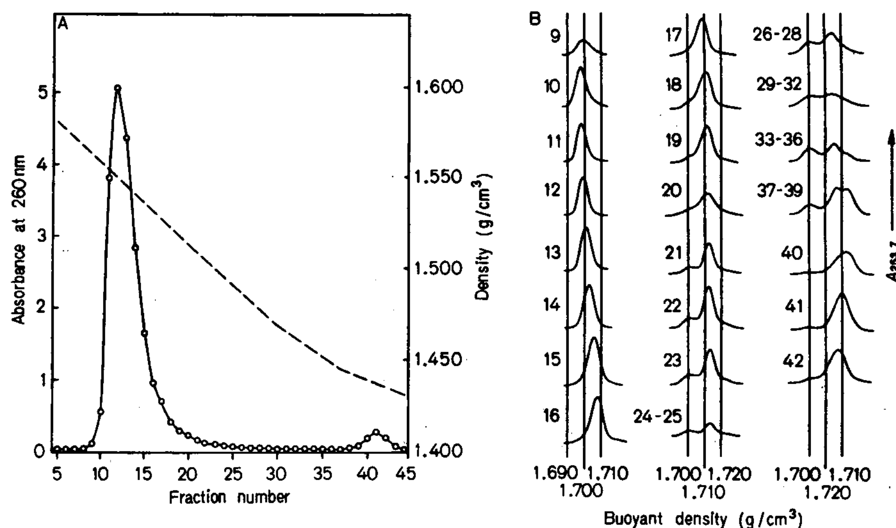

Fig. 1. *Fractionation of chicken DNA in Cs_2SO_4/Ag^+ density gradient at* $R_F = 0.40$. Chicken DNA (10 A_{260} units) in 5 mM $Na_2B_4O_7$, pH 9.2, containing Ag^+ ($R_F = 0.40$) and Cs_2SO_4 (ϱ = 1.50g/cm^3) was centrifuged in a Beckman type 30 rotor at 25 °C for 110 h at 25000 rev./min. (A) (○——○) Absorbance profile of the centrifuged DNA solution; (----) the density gradient. (B) Analytical CsCl profiles of individual fractions

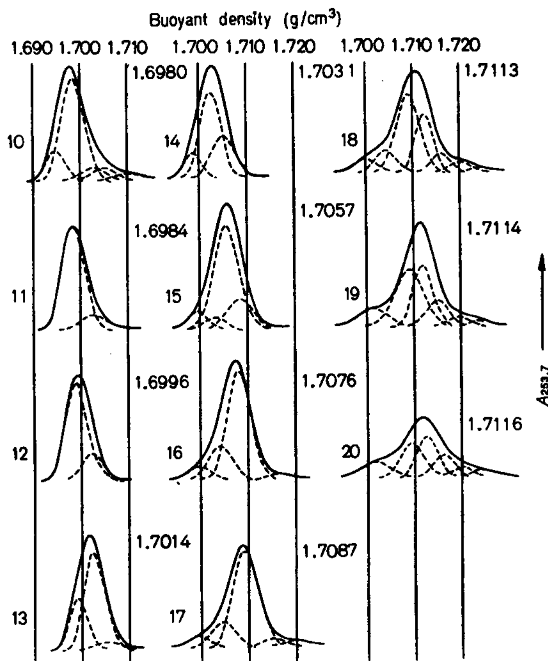

Fig. 2. *Gaussian analysis of CsCl profiles of Fig.* 1. The CsCl profiles of fraction 10–20 of Fig. 1 as analyzed in terms of gaussian components. The modal buoyant densities of the fractions are indicated

of the gradient and ending in a small peak close to the top of the tube. Each DNA fraction was analyzed by centrifugation in CsCl (Fig. 1 B). The analytical CsCl profiles were resolved into gaussian curves. Fig. 2 shows the results obtained for fractions 10–20; these fractions contain 90% of the DNA. The results of Fig. 1 B indicate the existence in the Cs_2SO_4/Ag^+ fractions of DNA components characterized by distinct modal buoyant densities. The gaussian analysis of the analytical profiles (Fig. 2) provided a precise estimate of the buoyant densities and relative amounts of the DNA components which were present in each fraction. Table 1 presents these results. Four major components were found to form 88% of chicken DNA; they had buoyant densities equal to 1.699, 1.702_5, 1.704_5 and 1.708 g/cm³, respectively. Seven minor and/or satellite components were also identified and characterized. (Note that we distinguish here, as in a recent paper [14], satellite and minor components according to their nucleotide sequence patterns and relative amounts in the genome as follows. Satellite components are formed by short repeated nucleotide sequences; each satellite component usually represents a small percentage of the genome. Minor components each account for less than 3% of the genome; they are not formed by short repeated nucleotide sequences, although they may contain a certain amount of these; several, if not all, minor components are formed by repeated genes.)

Fig. 3 A presents the absorbance profile of chicken DNA as centrifuged in a preparative Cs_2SO_4/BAMD

Table 1. *DNA components of the chicken genome*
The error in the buoyant density was ± 0.0005 g/cm³ [2]. The relative amount determined by Cs_2SO_4/BAMD gradients was estimated from the gaussian analysis of individual and of pooled fractions. Minor components were mainly present in the following fractions of Fig. 3: the 1.695-g/cm³ component in fractions 17–26; 1.701-g/cm³ in fractions 23–26; 1.706-g/cm³ in fractions 26–30 and 31–33 (this bimodal distribution may indicate the existence of two different components having the same buoyant density in CsCl); 1.7115-g/cm³ in fractions 23–28; 1.715-g/cm³ in fractions 28–33; 1.720-g/cm³ in fractions 27–33; 1.724-g/cm³ in fractions 26–32. Some of the minor components were detected by gaussian analysis of fractions shown in Fig. 4; other ones were found in fractions whose gaussian analysis is not shown in this paper; the corresponding pools are, however, shown in Fig. 5

	Buoyant density in CsCl	Amount determined by		
		Cs_2SO_4/Ag^+	Cs_2SO_4/BAMD	
			individual fractions	pooled fractions
	g/cm³	%		
Major components		88.6	87.5	87.3
	1.699	39.2	39.4	38.5
	1.702_5	25.4	24.3	24.4
	1.704_5	15.7	14.3	14.9
	1.708	8.3	9.5	9.5
Minor components		11.4	12.5	12.7
	1.695	0.4	0.4	0.3
	1.701	1.9	1.3	1.4
	1.706	0.4	0.4	0.4
	1.711_5	2.4	3.5	3.0
	1.715	3.2	3.0	3.5
	1.720	2.0	2.8	2.7
	1.724	1.1	1.0	1.4

density gradient at an $R_F = 0.14$. Under these conditions, 36% of the DNA was pelleted; the remaining DNA was spread over the whole density gradient. The absorbance profile was characterized by a main peak with two shoulders on the light side and a sharp light peak. The fractions from the preparative gradient and the pelleted DNA were analyzed by centrifugation in CsCl (Fig. 3 B and 5). These data confirm the existence of DNA components having different modal buoyant densities in the fractions of the preparative experiment. Because of the higher resolution of the Cs_2SO_4/BAMD gradient, the separation of different components was more clearcut than in the fractions of the Cs_2SO_4/Ag^+ gradient.

The analytical CsCl profiles were resolved into gaussian curves. Fig. 4 shows the results obtained for fractions 6–17 and Fig. 5 the results obtained for the pelleted DNA. The DNA components were thus assessed in terms of buoyant density and relative amounts (Table 1) and showed a very satisfactory agreement with those derived from the Cs_2SO_4/Ag^+ fractionation. On the basis of the results of Fig. 4, fractions were pooled and analyzed once more in terms of gaussian

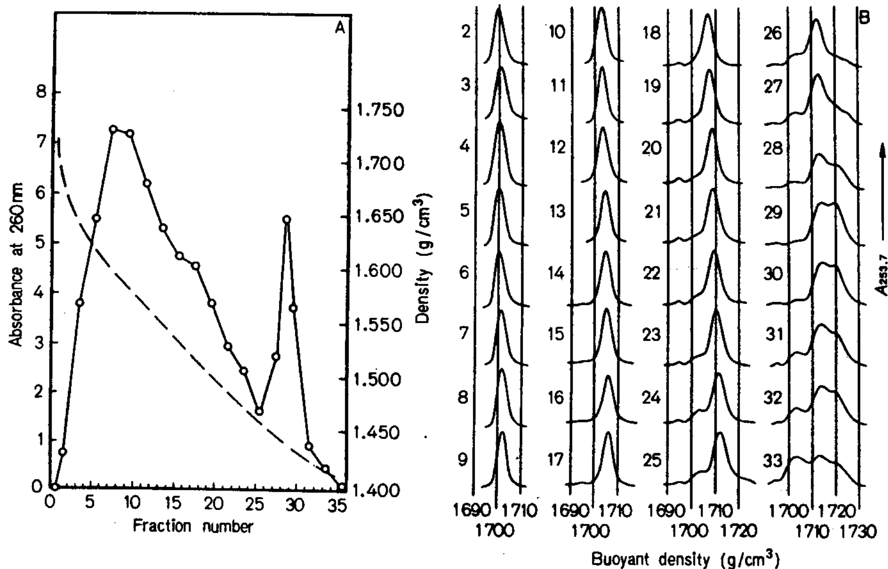

Fig. 3. *Fractionation of chicken DNA in* Cs_2SO_4*/BAMD density gradient at* $R_F = 0.14$. Chicken DNA (40 A_{260} units) in 0.1 M Na_2SO_4, 5 mM $Na_2B_4O_7$, pH 9.2, containing BAMD ($R_F = 0.14$) and Cs_2SO_4 (ϱ = 1.47 g/cm³) was centrifuged in a Beckman type 65 rotor at 25 °C for 60 h at 35000 rev./min. (A) (O——O) Absorbance profile of the centrifuged DNA solution; (----) density gradient. (B) Analytical CsCl profiles of individual fractions

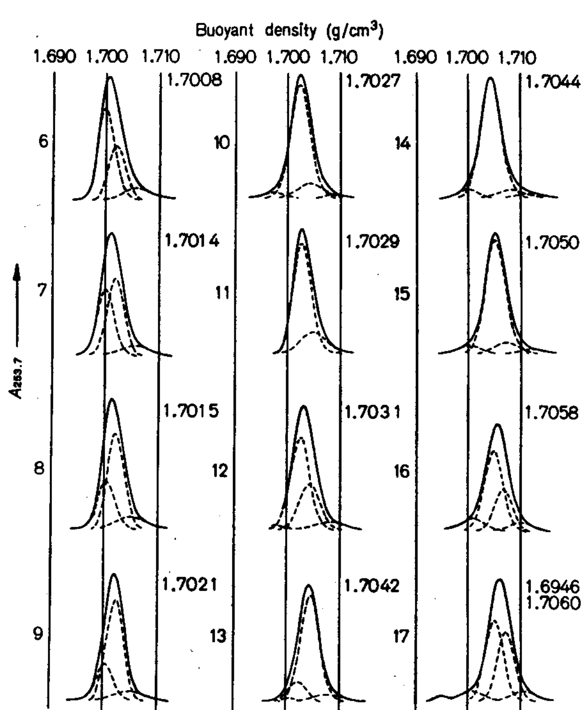

Fig. 4. *Gaussian analysis of CsCl profiles of Fig. 3.* The CsCl profiles of fractions 6–17 of Fig. 3 as analyzed in terms of gaussian components. The modal buoyant densities are indicated

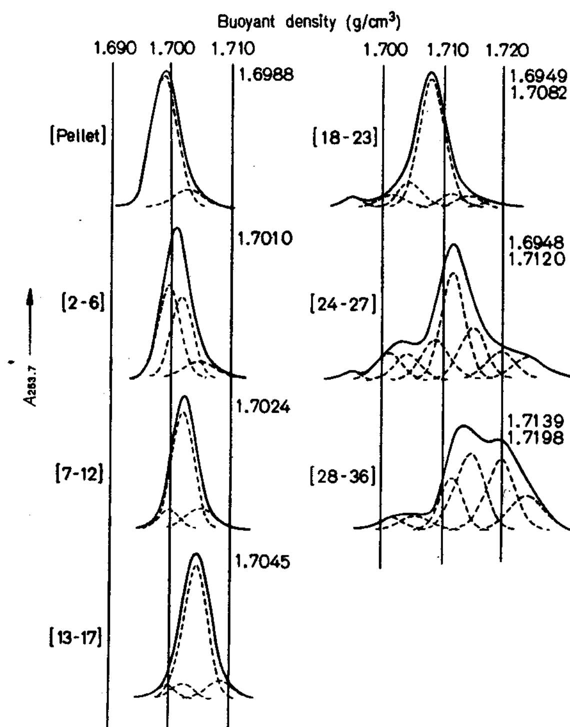

Fig. 5. *Gaussian analysis of CsCl profiles of pooled fractions.* The CsCl profiles of pooled fractions of the Cs_2SO_4/BAMD profile of Fig. 3 as analyzed in terms of gaussian components. The modal buoyant densities are indicated

curves (Fig. 5). The buoyant densities and relative amounts of the DNA components, as estimated in this way, are given in Table 1 and agree with the other estimates.

The Cs_2SO_4/BAMD procedure is of interest not only because of its usefulness in the analysis of the genome, but also for two other reasons. (a) By increasing the R_F value, an increasing amount of DNA can be pelleted and a higher resolution of dG + dC-rich components can be attained [5,8]. Fig. 6 shows the results of a preparative centrifugation in Cs_2SO_4/BAMD density gradient carried out at an R_F of 0.22.

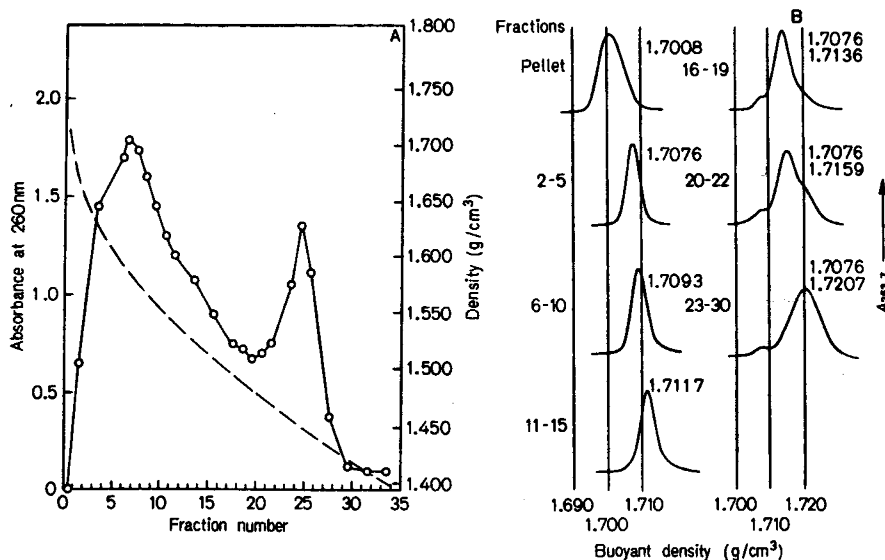

Fig. 6. *Fractionation of chicken DNA in Cs_2SO_4/BAMD density gradient at* $R_F = 0.22$. (A) (O——O) Absorbance profile of the centrifuged DNA solution; (----) density gradient. (B) Analytical CsCl profiles of pooled fractions. The modal buoyant densities are indicated

As expected, a larger amount of DNA, 85%, was pelleted under these conditions and a better resolution of dG + dC-rich components was observed; in particular, the 1.708 – g/cm³ component could be obtained in a highly purified form in just one centrifugation. (b) The Cs_2SO_4/BAMD centrifugation is of great value if one desires not only to analyze, but also to prepare, the DNA components; in fact, an additional centrifugation in Cs_2SO_4/Ag^+ of the pooled fractions of Fig. 5 yields DNA components in an essentially pure form (unpublished results).

Fig. 7 shows the hybridization of the ovalbumin gene probe on the pooled fractions of Fig. 5 corresponding to the major DNA components of the chicken genome, after degradation of the latter by restriction endonucleases *Hin*dIII and *Eco*RI. Four *Eco*RI fragments, 10000, 2200, 1750 and 1300 base pairs in size, and four *Hin*dIII fragments, 4700, 3200, 2200 and 1200 base pairs in size, from different pools showed hybridization with the probe. These results are in good agreement with those of Breathnach et al. [9], except for the fact that the 1200-base-pair *Hin*dIII band was not detected by those authors. Although the hybridization pattern is qualitatively the same for different pooled fractions, strong quantitative differences are evident (Table 2). The maximal hybridization was found for pooled fractions 7 – 12, where the 1.702_5-g/cm³ component is present to the extent of 77%. In the other pooled fractions, hybridization shows an excellent correlation with the amount of 1.702_5-g/cm³ component present in the pool.

It should be noticed that the relatively high level of hybridization in the pool of fractions 2 – 6 is due to the fact that the steep slope of the gradient in the bottom region of the tube causes a high local concentration of DNA and decreases the resolution of the lightest component, at 1.699 g/cm³, from the 1.702_5-g/cm³ component. In contrast the fractions 13 – 17, containing mainly the 1.704_5-g/cm³ component, show a very low amount of contamination by the 1.702_5-g/cm³ component, detected by both gaussian analysis and hybridization, owing to the better resolution of DNA components in this region of the gradient.

DISCUSSION

A comparison of the DNA fractionations obtained in Cs_2SO_4/Ag^+ and Cs_2SO_4/BAMD density gradients is of interest for several reasons. (a) The results of Fig. 1 and 3 show that centrifugation in the presence of BAMD has a higher resolving power than that in the presence of Ag^+. In the case of BAMD, the DNA was spread all over the gradient, instead of being concentrated in one relatively narrow band. In addition, as shown in Fig. 6, using a higher R_F value leads to pelleting a larger amount of dA + dT-rich DNA components and to a better resolution of the dG + dC-rich components left in the gradient. As already shown in the case of the fractionation of bovine satellite DNAs [5, 11], this effect is in part due to a decrease in the amount of DNA present in the gradient and in part to a different binding of BAMD by different dG + dC-rich components. (b) Fig. 3 A shows that the main DNA peak in a Cs_2SO_4/BAMD density gradient presents two shoulders on its light side. These shoulders are due to the presence of two DNA components, the 1.704-g/cm³ and the 1.708-g/cm³ components in fractions 13 – 17 and 18 – 21 respectively, as shown by the CsCl analysis. In other words, the existence of discontinuities in the distribution of buoyant densities in the bulk of chicken DNA, as shown by the ab-

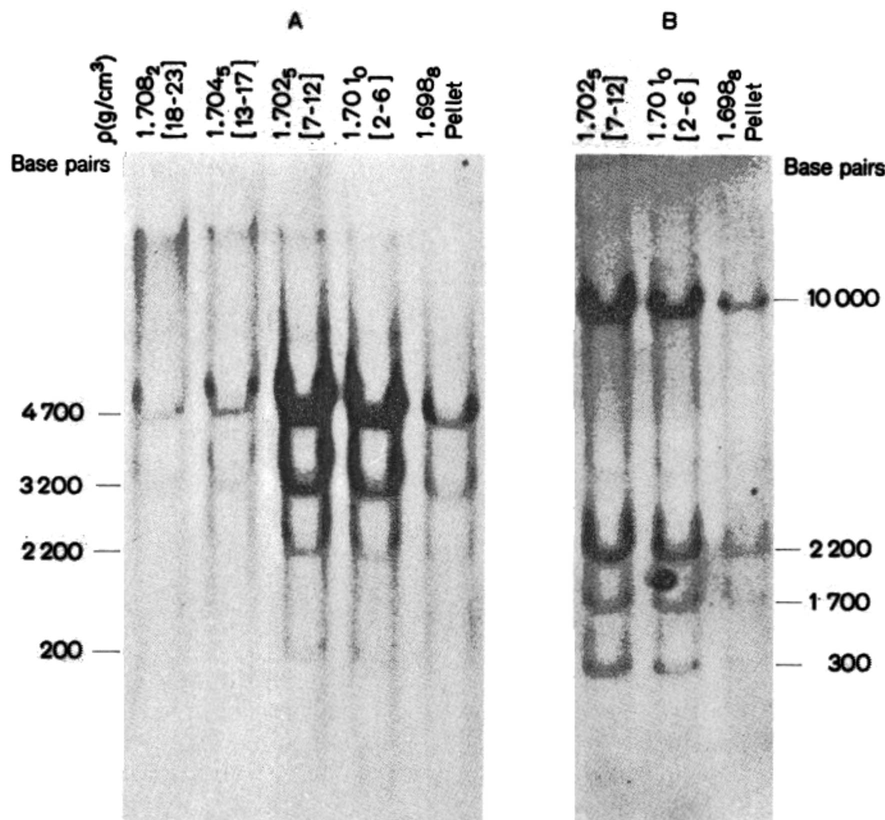

Fig. 7. *Detection of the ovalbumin gene in different pooled fractions of Fig. 5.* Aliquots of the density gradient pooled fractions of Fig. 5 (13 μg in every case) were digested to completion by restriction endonucleases (A) *Hin*dIII and (B) *Eco*RI, and electrophoresed on a 1% agarose gel. The hybridization with the ^{32}P-labelled *Hha* ov probe was carried out as described in Materials and Methods. The pooled fractions are indicated by their CsCl buoyant densities (ϱ), the size of the detected fragments are indicated by the number of residues

Table 2. *Relative amounts of the 1.702$_5$-g/cm^3 component and of the ovalbumin gene in chicken DNA fractions*
The amount of the 1.702$_5$-g/cm^3 component was estimated from the gaussian analysis of Fig. 5. The amount of hybridization was estimated by measuring the intensity of hybridization bands relative to that of the bands shown by the pooled fractions 7–12. A Joyce-Loebl microdensitometer (Newcastle-on-Tyne, U.K.) was used to scan the autoradiographs. The hybridization shown by fractions 7–12 was made equal to 77 for an easier comparison with the amount of 1.702$_5$-g/cm^3 component. b.p. = base pair

Fraction	Amount of 1.702$_5$-g/cm^3 component	Relative hybridization of ovalbumin probe to fragments		
		*Hin*dIII 4700-b.p. band	*Hin*dIII 3200-b.p. band	*Eco*RI 10000-b.p. band
	%			
Pellet	11	8	8	8
Pooled fractions:				
2–6	42	45	51	39
7–12	77	77	77	77
13–17	7	5	6	6
18–23	5	4	3	4

sorbance profile of Fig. 3 A, reflect the transitions from one major component to the following one. (c) Owing to the high resolution of Cs_2SO_4/BAMD gradients, fractions containing essentially pure DNA components were obtained already on a first run, whereas in the case of Cs_2SO_4/Ag^+ gradients all fractions represented a mixture of different DNA components. The gaussian analysis of the CsCl profiles shown in Fig. 2 and 4 demonstrate this point. In fact, the resolving power of Cs_2SO_4/BAMD gradient is such that the same component is found in several subsequent fractions and a fine resolution of the small internal compositional heterogeneity of individual components can be obtained. For instance, the 1.702$_5$-g/cm^3 component is found in fractions 8–11 of Fig. 4, the 1.704-g/cm^3 component in fractions 13–15 of Fig. 4, and the 1.708-g/cm^3 component in fractions 2–5 of Fig. 6; the modal density of DNA fractions in each series slightly increases with fraction number. Such a slight, continuous increase in the buoyant density of fractions containing essentially a pure component levels off at the transition between two components and is followed by a discrete jump in modal buoyant density corresponding to the

appearance of the next DNA component (Fig. 3B and 4). (d) BAMD fractions at the border of two subsequent components are characterized by the simultaneous presence of both components and by a band which is larger than either the preceding or the following fractions. For instance. fraction 12, at the border of the 1.702_5-g/cm^3 and 1.704-g/cm^3 components (Fig. 4), contains both components and the band width of its CsCl peak is larger than those of fractions 11 and 13. This increase in band width precedes the jump in buoyant density mentioned above. (e) In spite of their different resolving power, the two methods used lead to estimates of buoyant densities and relative amounts of all DNA components which are in good agreement (Table 1). This shows that the gaussian analysis performed on both series of analytical profiles provides a precise estimate of different DNA components regardless of the number and relative amounts of the components in a given fraction. A comparison of the results obtained by analysing the CsCl profile of total chicken DNA [2] with those obtained here revealed that although the estimates obtained with the former method provided a general idea of the components present, only the more refined approaches involving DNA ligands could lead to precise values for both the modal buoyant densities and the relative amounts of the DNA components.

The distribution of DNA fragments carrying the ovalbumin gene in the Cs_2SO_4/BAMD fractions exactly follows the distribution of the 1.702_5-g/cm^3 component (Table 2). This proves that the gene is carried by DNA fragments belonging to this component. The chromosomal segment represented by the population of overlapping fragments containing the gene has an M_r as large as 10^8, namely 25 times larger than the smallest fragment containing all the coding sequence (4×10^6 [10]). This indicates the homogeneity of base composition over chromosomal segments with M_r as large as 10^8, in agreement with previous evidence [3] that DNA components correspond to large segments of homogeneous base composition. The localization of the ovalbumin gene on the 1.702_5-g/cm^3 component is the second case in which a gene has been so localized, the first one being that of the β-globin gene in the 1.701_5-g/cm^3 component in the mouse genome [11]. Other genes have been localized more recently in the major components of mammalian genomes [12].

As far as the organization of the chicken genome is concerned, the present work shows that it is mainly made up of major DNA components which are similar in relative amounts and buoyant densities to those previously investigated in mammals [1–3]. Again, four major components are found; the two light ones represent the majority of the genome and correspond in buoyant density to the DNAs of lower vertebrates; the two heavy ones, absent in lower vertebrates, are mainly responsible for the skewness of chicken DNA on the heavy side of the CsCl band. This similarity is of interest because of the common evolutionary origin of mammals and birds and raises the point of a correspondence of the genetic information carried by the corresponding DNA components of mammalian and avian genomes. Since the haploid genome size of birds is only about half that of mammals [13], it will be interesting to compare avian and mammalian DNA components as far as the renaturation kinetics are concerned to see how much of the differences is accounted for by interspersed repetitive sequences.

Seven minor and/or satellite components were also found in these investigations. Three of these components (at 1.695, 1.701 and 1.706 g/cm^3) showed an anomalous behavior in the preparative density gradients, in that they banded in Cs_2SO_4/BAMD gradient at densities different from those expected on the basis of their buoyant density in CsCl (see last column of Table 1); such behavior is indicative of the presence of short repetitive sequences in these components [14]. Two of the minor and/or satellite components seen in this work (at 1.706 and 1.711_5 g/cm^3) may be identical to components detected by Colbert et al. [15] in the same breed of chicken; the other components reported by these authors (at 1.689, 1.697, 1.703 and 1.710 g/cm^3) were not seen here.

Finally, it should be mentioned that the existence of DNA components in mammalian and avian genomes has been recently questioned by Vizard et al. [16] on the basis of electron microscopy and thermal denaturation results obtained on human DNA. We will show elsewhere that the results of Vizard et al. [16] have been interpreted incorrectly and that, in fact, they do not contradict the existence of DNA components.

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Note Added in Proof. Hybridization of the ovalbumin probe on isolated DNA components from chicken has confirmed that the gene is localized in the 1.702_5 g/cm^3 component.