Eur. J. Biochem. *130*, 241–245 (1983)

Organization of Nucleotide Sequences in the Chicken Genome

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(Received September 30, 1982) – EJB 6054

The four major components of chicken DNA were prepared by density gradient centrifugation and characterized in several basic properties: relative amounts, dG + dC content, buoyant densities, compositional heterogeneity, and reassociation kinetics. While the relative amounts and the compositions of the major components of chicken DNA were similar to those found in mammalian genomes, their compositional heterogeneities were found to be narrower. The relative amounts of interspersed repeated and unique sequences were strikingly different in different components and also different from those found in the corresponding major components of mouse and human DNAs. If one takes into consideration that major DNA components (a) account for practically all of main-band DNA and (b) derive by preparative breakage from very long DNA segments of fairly homogeneous composition, the isochores, our findings indicate that the distribution of interspersed repeats is different in different chromosomal regions and is species-specific.

Equilibrium density gradient centrifugation in the presence of DNA ligands has been applied in our laboratory to investigate the nucleotide sequence organization of the genomes of eukaryotes and, more particularly, of vertebrates [1–8]. This approach has led us, so far, to several conclusions. The first one is that, neglecting satellite and minor components, the genomes of higher, warm-blooded vertebrates can be resolved into four families of fragments, the major DNA components. Two of these components (not resolved in some species) represent about two thirds of the main-band DNA and have densities in the range 1.697–1.702 g/cm^3, whereas the other two represent about 25% and 10% and have densities of 1.704 and 1.708 g/cm^3, respectively. Both the relative amounts and the buoyant densities of the major components show little variation in different mammalian and avian genomes. In contrast, DNAs from lower, cold-blooded vertebrates have buoyant densities which are, in most cases, in the same range as the light components of warm-blooded vertebrates; these DNAs do not show the compositional heterogeneity of DNAs from higher vertebrates and cannot be resolved into families of fragments equivalent to the major components mentioned above.

The second conclusion is that the four major components of warm-blooded vertebrates are families of DNA fragments derived, by preparative breakage, from very long DNA segments (3×10^5 residues in the mouse genome) fairly homogeneous in base composition, the isochores, possibly corresponding to chromosomal bands. Furthermore, unique, interspersed repeated and foldback sequences show a different distribution in the major components of mouse and human DNAs, and also in corresponding major components of these mammalian DNAs. The first finding points to a sequence interspersion pattern which varies in different chromosomal regions; the second one to an unsuspected variety of sequence arrangements in mammals.

In the present work we have investigated the basic properties and the nucleotide sequence organization of the major components of the chicken genome. These investigations follow up an analytical examination [6] and lead to the conclusions that (a) the distribution of repeated and unique sequences in the chicken genome shows a regional variation as previously found in the mammalian genomes and (b) that this distribution is different from those found in the corresponding major components of mammalian DNAs.

MATERIALS AND METHODS

Preparation of the Major Components of the Chicken Genome

The DNA preparation used as the starting material in this work was obtained from the erythrocytes of a male White Leghorn chicken. This preparation was described previously [6] and its molecular weight was 57×10^6. The major components of chicken DNA were prepared using three density gradient centrifugations.

The first centrifugation was performed in a Cs_2SO_4/BAMD density gradient [4] at pH 9.2 and at an r_f (the ligand/nucleotide molar ratio) of 0.14, a choice suggested by our previous analytical work [6] to provide optimal resolution of the major components of chicken DNA. Under the DNA load and the experimental conditions used, 47% of chicken DNA, was pelleted, the rest being spread over the whole gradient (Fig. 1). Because of differences in both DNA load and centrifugation conditions, the distribution was not the same as that reported previously (see Fig. 3 of [6]). Analytical CsCl centrifugation showed that the pellet essentially corresponded to the lightest major component (Fig. 2A); fractions 1–19 in the gradient were pooled on the basis of several criteria (modal buoyant densities, band widths, asymmetries) and of the previous gaussian analysis [6], to produce three pools (2, 3 and 4; Fig. 2A) basically corresponding to the other three components. The subsequent fractions, 20–40, representing minor and satellite components [6], were not processed any further. Because of the high resolving power of Cs_2SO_4/BAMD gradients and the high molecular weight of the DNA preparation used, some of the fractions at this stage contained essentially pure DNA components, as shown by analytical

Abbreviations. BAMD, bis(acetato-mercurimethyl)dioxane; r_f, ligand/nucleotide molar ratio.

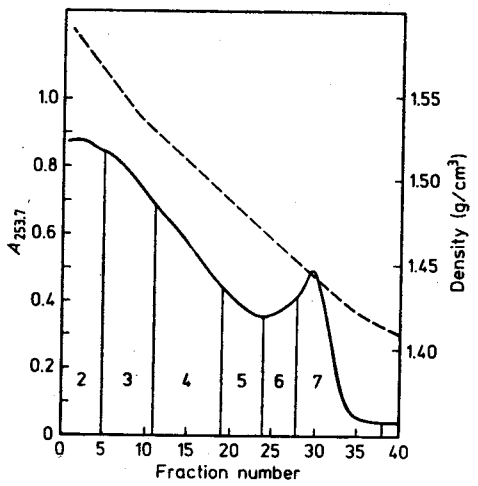

Fig. 1. *Fractionation of chicken DNA in Cs_2SO_4/BAMD density gradient at $r_f = 0.14$.* Chicken DNA (111 A_{260} units) was centrifuged in 0.1 M Na_2SO_4, 5 mM $Na_2B_4O_7$, pH 9.2, containing BAMD (r_f = 0.14) and Cs_2SO_4(ϱ = 1.47 g/cm³) using a Beckman type 30 rotor at 25 °C for 113 h at 25000 rev./min. (——) Absorbance of DNA at 253.7 nm; (– – – –) density gradient. Pool numbers are shown, 1 corresponds to the pellet

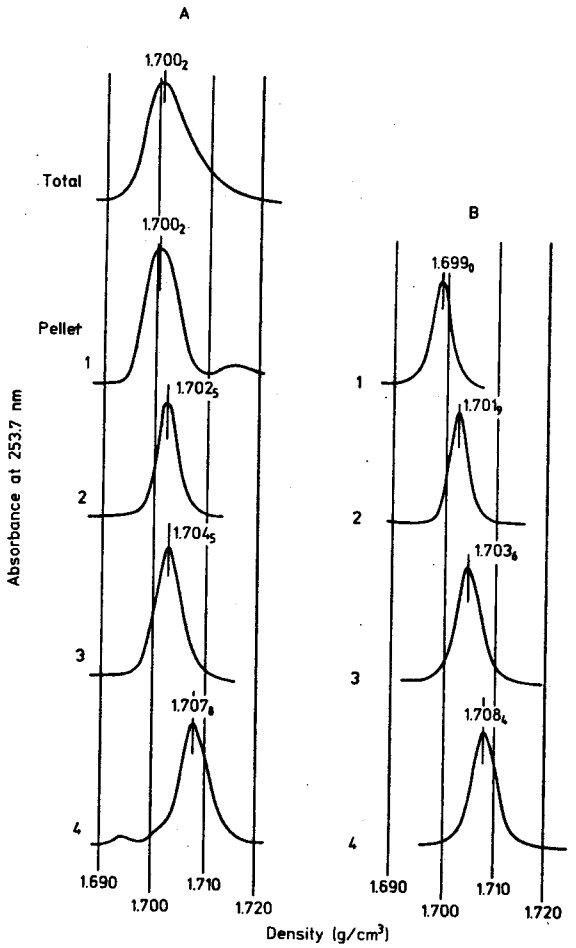

Fig. 2. *Analytical CsCl profiles.* (A) Total unfractionated chicken DNA and fractions from the Cs_2SO_4/BAMD density gradient (Fig. 1). (B) Purified major components from the four Cs_2SO_4/AG^+ gradients

CsCl centrifugations, which were routinely done on all fractions, together with determination of sedimentation coefficient.

The four fraction pools from the first centrifugation were centrifuged again in CsCl gradients having initial densities chosen as to provide optimal resolution of each pool, but the degree of further enrichment achieved by this step was very small. The third centrifugation was carried out in Cs_2SO_4/Ag^+ gradients at pH 9.2 [6]; r_f values of 0.30, 0.30, 0.33 and 0.35 were used to obtain optimal resolution of components 1–4, respectively, based on data obtained by analytical centrifugation. The central fractions were again pooled to yield the preparations of major DNA components used in these studies. They exhibited the CsCl analytical profiles shown in Fig. 2B.

The final yield of major components was estimated to be about 80% of the DNA obtained from fractions 1–19 of the first centrifugation.

Other Materials and Methods

Renaturation kinetics was investigated as described elsewhere [9] except for the following. In the range of c_0t values (c_0 being the initial DNA concentration, t the incubation time) lower than 10 M · s, dilute DNA solutions (approximately 50 µg/ml) in 0.03–0.04 M potassium phosphate and different KCl concentration were used. DNA samples (2 µg) were sealed in capillaries, heated for 10 min in boiling water and incubated at a temperature 25 °C below the melting temperature for various times. As the first-order renaturation of inverted repeated sequences requires a non-negligible time [10], reassociations at c_0t of 10^{-4} M · s were done for 60 s.

The melting temperatures of the major components and of unfractionated chicken DNA were determined for each different buffer concentration by using the following relationship (G. Cuny, personal communication): melting temperature = (80.6 ± 0.43) + (0.395 ± 0.08) (dG + dC) + 14.64 log [K^+]°C, which is in good agreement with the relationship of Gillis et al. [11].

The reassociation profiles, namely the curves of the fraction of reassociated DNA vs 'equivalent' c_0t were resolved into second-order kinetic curves, using a Fortran program made for a Control Data 6600 computer as already described [9]. The solution retained in this trial-and-error procedure was the one fitting the data with a minimum number of kinetic classes and with the smallest total least-square deviation from the experimental values.

Sedimentation velocity, base composition, intermolecular compositional heterogeneity were investigated as already described [8].

RESULTS

Basic Properties of the Major Components of the Chicken Genome

Table 1 presents the relative amounts, the molecular weights, the modal buoyant densities, the intermolecular heterogeneities and the base compositions (as determined by nucleoside analysis or as calculated from the buoyant densities) of the major components of chicken DNA. The relative amounts of each component, as estimated from preparative yields, were in excellent agreement with those previously obtained by gaussian analysis [6]. The modal buoyant densities of the isolated major components also agreed with estimations made in our previous analytical work [6]. The CsCl band profiles of the major components were highly symmetrical (Fig. 2B), with mean buoyant densities, $\langle\varrho\rangle$, identical to modal buoyant densities, ϱ_0. This is in sharp contrast to the asymmetry of the profile of total chicken DNA (Fig. 2A; $\langle\varrho\rangle - \varrho_0 = 2.2$ mg/cm³).

Table 1. *Basic properties of chicken DNA and its components*
ϱ_0 is the modal buoyant density in CsCl; the error is ± 0.0005 g/cm^3. H is the intermolecular heterogeneity given as a percentage of dG + dC. The percentage of dG + dC content of DNA was calculated from ϱ_0 values according to the relationship of Schildkraut et al. [12] or determined by nucleoside analysis; the error is $\pm 0.5\%$. The molecular weight, M_r, is calculated from the sedimentation coefficient s according to the relationship of Eigner and Doty [13]

DNA	Amount	M_r $\times 10^{-6}$	ϱ_0	H	dG + dC	
					calculated	determined
g/cm^3	%		g/cm^3	%		
Total	100	57	1.700_2	5.1	41.0	39.3
1.699	39	57	1.699_0	1.7	39.8	38.3
1.702	25	45	1.701_9	2.1	42.8	39.7
1.705	15	46	1.703_6	2.5	44.5	42.7
1.708	9	57	1.708_4	2.9	49.4	46.7

Table 2. *Kinetic classes in chicken DNA and its major components*

DNA	Amount	Class	Amount of class	$c_0t_{1/2}$
g/cm^3	%		%	M · s
Total	100	foldback	3	–
		very fast	3	0.02
		intermediate	10	5
		slow	84	1050
1.699	39	foldback	3	–
		fast	5	0.2
		intermediate	5	6
		slow	87	900
1.702	25	foldback	2	–
		fast	5	0.15
		intermediate	10	5
		slow	83	570
1.704	15	foldback	3	–
		fast	5	0.1
		intermediate	2	1.4
		slow	90	447
1.708	9	foldback	4	–
		fast	5	0.2
		intermediate	17	1.5
		slow	74	190

The estimates of dG + dC contents of the major components as calculated from ϱ_0 values were systematically higher than those obtained by direct nucleoside analysis. This discrepancy was also found for the major components of mouse and human DNAs [8] and is apparently due to a different frequency of short oligonucleotide sequences in these vertebrate DNAs compared to the bacterial DNAs used in establishing the dG + dC vs ϱ_0 relationship. Finally, the compositional heterogeneities (Fig. 3) of the isolated major components were remarkably small not only when compared with that of total chicken DNA, but also with the values for the major components of mouse and human DNAs [8].

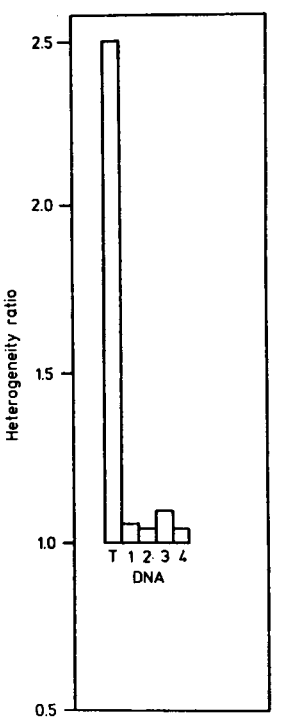

Fig. 3. *Histogram of heterogeneity ratios for total chicken DNA (T) and the four major components (marked 1–4 in order of increasing density).* The heterogeneity ratio is the result of dividing the heterogeneity of the chicken DNA by that of a bacterial DNA having the same molecular weight and the same dG + dC content. (Results taken from Cuny et al. [8])

Reassociation Kinetics

Fig. 4 shows the reassociation kinetic profiles obtained with total chicken DNA and its major components. Table 2 presents the relative amounts and the equivalent $c_0t_{1/2}$ values (as defined by Britten et al. [14]) of the different kinetic classes as present in total DNA and its major components.

The results obtained with total DNA indicate a very high proportion (84%) of slowly renaturing unique sequences; sequences in the intermediate and foldback classes account for a total of 13% of the DNA, while sequences in the very fast class account for 3%. The isolated major DNA components show distinctly different kinetic profiles. In the first place one notes the absence of the very rapidly renaturing material ($c_0t_{1/2}$ = 0.02 M · s) observed in total DNA. This probably represents satellite DNA eliminated during purification; satellites have been shown previously to account for 3% of chicken DNA [6]. Secondly, the proportion of material in the intermediately renaturing class varies widely among the four components, with values as low as 2% in the 1.704-g/cm^3 component and as high as 17% in the 1.708-g/cm^3 component. These differences are accompanied by opposite shifts in proportion to slowly renaturing material without any change in other classes.

Examination of the $c_0t_{1/2}$ values indicates a decline in complexity for unique sequences, when going from the lightest to the heaviest component. This is expected in view of the relative amounts of DNA (39%, 25%, 15% and 9%) in the four components from the lightest to the heaviest. Intermediately renaturing material also shows reduced $c_0t_{1/2}$ values in the two heaviest components. Finally, all four components show 5% of the DNA in a rapidly renaturing class ($c_0t_{1/2}$ = 0.1–0.2 M · s). This material is not resolvable

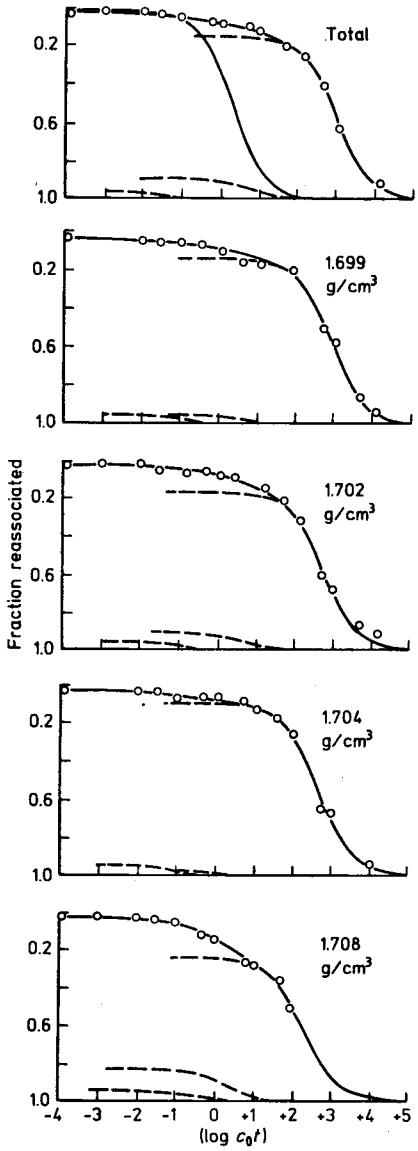

Fig. 4. *Reassociation kinetics of chicken total DNA and the four major chicken components.* Results with *Escherichia coli* DNA (——) are shown for comparison. The solid lines through the experimental points (O——O) are the overall profiles resulting from the analysis of kinetic classes (- - - -) described in Materials and Methods. c_0t values are given in mol nucleotide · l^{-1} · s

from the intermediately renaturing class of total, unfractionated, DNA.

DISCUSSION

The haploid chicken genome consists of only 1.1 – 1.4 pg of DNA, a value less than half that of mammalian genomes. In spite of this considerable difference, native chicken DNA can be fractionated into major components which are similar, in both their buoyant densities and relative amounts, to those of mammalian DNAs. Chicken total DNA, like human DNA [8], shows a unimodal profile in CsCl, without satellite bands, that is skewed on the heavy side. The skewness is due to the preence of two heavy major DNA components, which represent together 24% of the DNA, and of seven minor components and cryptic satellites accounting for 12% of the DNA, most of which range in density from 1.706 to 1.724 g/cm³ [6]. The high yield of major components and the excellent agreement between their relative amounts as judged from preparative yields and gaussian analysis [6] suggest that, as in the case of mammalian genomes [8], major components account for nearly all the chicken DNA.

The compositional heterogeneity of chicken total DNA is greater than that of human DNA, apparently because of the large amount of heavy minor and satellite components. However, the isolated major components from chicken DNA are significantly more homogeneous, in terms of composition, than those from mammalian DNAs [8]. In fact, compositional heterogeneity is almost identical to that of bacterial DNAs of the same size and base composition (Fig. 3). The result is even more striking when one considers the much greater complexity of the chicken major components, especially the light components, compared to bacterial DNAs. This implies a higher level of compositional homogeneity in the DNA fragments for the major components relative to bacterial DNAs.

Renaturation kinetics of total DNA obtained here are in agreement with previous reports [15, 16], showing a very large proportion of unique sequences (84%) with only 13% repetitive sequences. The results for the isolated major components, however, indicate a non-uniform distribution of the various kinetic classes in the chicken genome. The 1.708-g/cm³ component, in particular, shows a significant enrichment in repetitive sequences of the intermediately renaturing class, whereas these sequences are almost completely absent from the 1.704-g/cm³ component. In the latter case, this means that the isochores from which these large fragments of homogeneous composition are derived must consist almost entirely of unique sequences uninterrupted by interspersed repeats. Similarly, the low level of intermediately repeated sequences in the two light components does not allow a short-period interspersion pattern of the *Xenopus* type [17] to be significantly represented, whereas this is quite possible in the 1.708-g/cm³ component.

It is also of interest to compare these results with those obtained in mammals [9]. In the first place one notes, in the case of mouse DNA components, that the foldback and rapidly renaturing classes decrease in proportion from the lightest to the heaviest components and are totally absent in the 1.708-g/cm³ component. In the case of human DNA components, these kinetic classes, in contrast, increase in proportion from the lightest to the heaviest component. No such trend is shown in the chicken DNA components, where foldback and rapidly renaturing classes are stable in proportion throughout all four components. The 1.708-g/cm³ component, which shows the highest fraction of intermediate renaturing repeats in chicken (17%), shows values of 9% in mouse and 10% in man. When rapidly renaturing and foldback sequences are excluded, however, the relative amounts of intermediately repeated sequences in the 1.708-g/cm³ components of human (18%) and chicken (19%) DNAs are similar. Nevertheless, the intermediately repeated sequences are the most abundant in the two lightest components of the mouse DNA and in the 1.704-g/cm³ component of the human DNA. Interestingly, the different amounts of kinetic classes in the chicken DNA components are not reflected in their compositional heterogeneity nor in their overall base composition, indicating the same compositional properties for both intermediately and slowly renaturing sequences located in the same major component. Similar conclusions have already been reached by analyzing the base compositions of denatured and reassociated mouse DNA fractions [9].

In summary, these investigations extend our main conclusions on the sequence organization of two mammalian genomes [8,9] to the genome of chicken.

We thank Dr Jordi Cortadas for his help in some experiments and for useful discussions of the results reported here.

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