SATELLITE COMPONENTS OF CALF THYMUS DNA. CHROMATOGRAPHIC ISOLATION OF THE NATIVE COMPONENTS AND CHARACTERIZATION OF THEIR SEQUENCE HETEROGENEITY

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Calf thymus DNA was fractionated by chromatography on columns of methylated albumin-kieselguhr combined with partial denaturation and selective precipitation with histone F1. Some of the fractions obtained were characterized in a CsCl density gradient as homogeneous satellite components with densities of 1·707 g/cm^3, 1·714 g/cm^3 and 1·721 g/cm^3. The sequence heterogeneity of these components was further studied and all the three satellite components were shown to contain, besides repetitive sequences, also sequences with a very small ability of renaturation. In the presence of silver ions these satellite preparations homogeneous in density are separated in a Cs_2SO_4 density gradient into several components.

Although much effort has been spent on the study of satellite components of eukaryotic DNA's very little can be said with certainty about their function in the genome[1]. At present, it appears most likely that the function of these homogeneous and repetitive components is mainly in the chromosome folding or in influencing their mutual affinity, both these effects being generally known as the chromosomal housekeeping function[1]. This possibility is supported by the frequently demonstrated localization of satellite sequences near the centromere areas of the chromosomes[2-5]. In connection with the possible molecular mechanism of such a chromosomal housekeeping function it is of interest to mention the recent findings of a selective interaction of histones with DNA of definite composition[6,7]. Changes of DNA conformation due to these interactions depending on the composition and possibly the sequence of DNA (ref.[8]) have also been reported.

For further study of these problems it would be of advantage to possess a method for isolating milligram amounts of well characterized satellite components from readily available and suitable material. Such material is represented by DNA isolated from calf thymus especially since the protein components of calf thymus chromatin have been thoroughly characterized[9]. For isolation methods one would look first of all to chromatographic separations.

Chromatography on methylated albumin – kieselguhr columns has already been used for isolating native fractions of DNA enriched with the satellite components[10-11]. For isolation of satellite components of calf thymus DNA rich in (G + C) combining this method with partial denaturation[12] appeared to have obvious advantage. Partial denaturation has already been used for similar purposes in combination with other separation techniques[13].

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Some properties of the satellite components of calf thymus DNA have been reported before[12,14–16]. In the present paper we describe a new isolation procedure and results obtained with components isolated without using preparative gradient centrifugation and we try to contribute to a more detailed characterization of various calf thymus DNA components.

EXPERIMENTAL

DNA *samples*. DNA was isolated according to Kay and coworkers[17]. The DNA concentration was determined from absorbance at 260 nm using the value $A^{1\%}_{1cm} = 200$. The molar concentration of DNA phosphate [P] was determined from $[P] = 1{\cdot}47 \,.\, 10^{-4} A_{1cm}$ (ref.[18]).

Fractionation of DNA. Fractions enriched with satellite components were obtained from calf thymus DNA by a two-step fractionation on methylated albumin — kieselguhr (MAK) columns according to Mandell and Hershey[19]. In the first step a total of 25—30 mg DNA was separated on a three-layer column 70 . 130 mm. The column contained two layers of kieselguhr coated with methylated albumin differing in the content of methylated albumin and a thin protective kieselguhr layer. DNA was adsorbed to the column from 0·525M-NaCl + 0·013M phosphate, pH 6·8, and eluted from the column with a linear gradient of NaCl (0·525M—0·9M) in 0·013M phosphate buffer. For further fractionation we used fractions eluted at lower NaCl molarities (20—30% of starting DNA). This fraction (pooled from two to four large columns, *i.e.* 25—30 mg DNA) was equlibrated with standard saline citrate (0·15M-NaCl + 0·015M trisodium citrate) and heated for 20 min at 94°C or 96°C. After cooling and adjusting the NaCl concentration to 0·525M it was fractionated on a methylated albumin — kieselguhr column. The separation procedure was the same as with the native sample. A total of 3—4 mg native DNA heavily enriched with satellite components has been obtained.

Further separation of the fractions enriched with the satellite components was done on columns 15 . 80 mm in size containing one layer of kieselguhr coated with methylated albumin and a thin protective layer of kieselguhr. The DNA was adsorbed to the column from 0·525M-NaCl + 0·013M phosphate, pH 6·8, and eluted with a linear gradient of NaCl (0·525M—0·9M) in 0·013M phosphate buffer.

Precipitation of satellite DNA *with histone* F1. DNA and histone F1 were mixed at a ratio of 2 : 1 in 2M-NaCl and the mixture was dialyzed succesively against 0·4M-NaCl, 0·3M-NaCl and 0·15M-NaCl. The insoluble complex of DNA with the F1 histone was then separated by centrifugation and DNA remaining in the supernatant was used for further measurements. The procedure is described in detail elsewhere[6].

Thermal chromatography of DNA was done according to Miyazawa and Thomas[20]. DNA, dissolved in 0·08M phosphate ($NaH_2PO_4 + Na_2HPO_4$, 1 : 1) was adsorbed to a column of hydroxyapatite 10 . 20 mm. The temperature was gradually raised at small intervals and DNA was eluted at each temperature with 15 ml of 0·08M phosphate. The temperature was measured with a copper–constantan thermocouple.

Melting curves were determined in a Hilger Uvispek spectrophotometer at 260 nm using 1 cm cells.

Equilibrium centrifugation in a CsCl *density gradient* was done in a Spinco Model E ultracentrifuge at 44770 rev./min for 20—24 h using cells of 12 mm optical path. The marker used was

Staphylococcus aureus DNA of a density of 1·693 g/cm^3. The densities were calculated according to Schildkraut and coworkers[21].

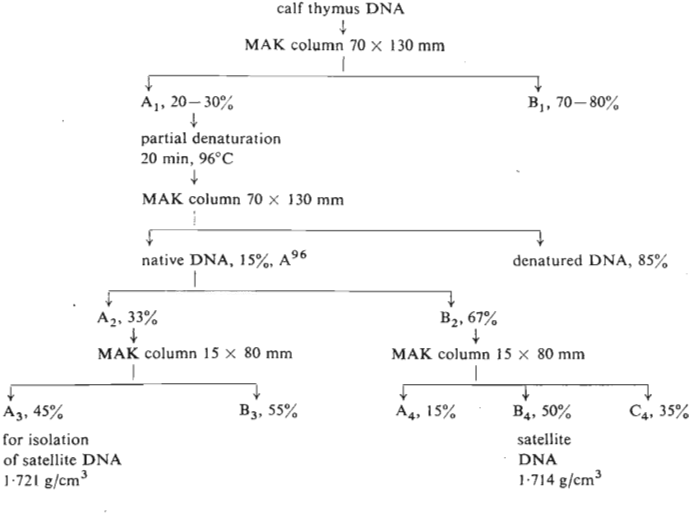
Equilibrium centrifugation in a Cs_2SO_4—Ag^+ *density gradient.* This method was carried out as described by Jensen and Davidson[22] and Filipski and coworkers[16]. DNA samples at a concentration 9—12 μg/ml were dialyzed against 5mM borate buffer of pH 9·2. 0·1mM $AgNO_3$ was added to reach the value of r (ratio Ag^+/DNA phosphate) equal to 0·35 and finally the density was adjusted to 1·5 g/cm^3 by addition of solid Cs_2SO_4. Centrifugation was done in a Spinco Model E ultracentrifuge at 44770 rev./min for 20—24 h in cells with an optical path of 12 mm.

Sedimentation coefficients were determined in a Spinco Model E ultracentrifuge in cells with an optical path of 30 mm. The DNA was dissolved in 0·9M-NaCl + 0·1M-NaOH. The molecular weight of single-stranded DNA was calculated according to Studier[23].

RESULTS

Isolation of Satellite Components of DNA *from Calf Thymus*

The fractionation procedure using partial denaturation is shown on Scheme 1. The first steps of this procedure were already used[12] for isolating a fraction enriched with



SCHEME 1

satellite DNA's. This fraction, designated in Scheme 1 as A^{96}, contains a mixture of 45% of the satellite componènt of density 1·714 g/cm^3, 20% satellite DNA of density 1·721 g/cm^3 and the remaining DNA of lower density[12]. As shown by the profiles of the CsCl density gradients of the individual fractions (Fig. 1) further fractionation on methylated albumin-kieselguhr columns permits to separate quantitatively the low-density DNA (it remains in fraction C_4) from the other fractions which represent mixtures of the two high-density satellite components. The fraction B_4 then represents practically homogeneous satellite component of density 1·714 g/cm^3 (the content of heavier and lighter components does not exceed 10%). The procedure indicated in Scheme 1 led to an at most 50% enrichment of fraction A_3 with the 1·721 g/cm^3 component.

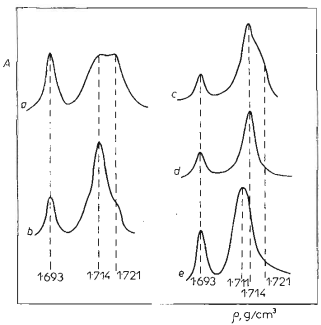


FIG. 1

CsCl Density Gradient Centrifugation of Calf Thymus DNA Fractions

The fractions were obtained as shown in Scheme 1 *a* fraction A_3; *b* fraction B_3; *c* fraction A_4; *d* fraction B_4; *e* fraction C_4.

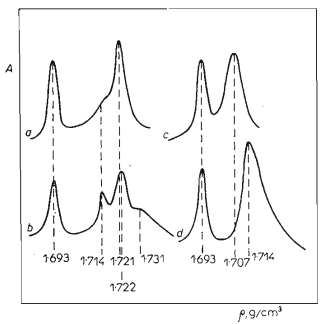
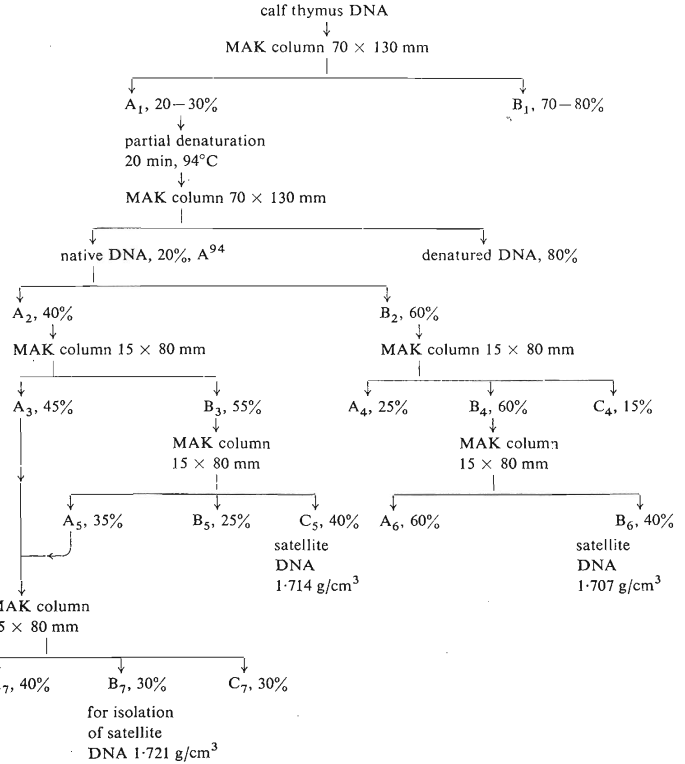


FIG. 2

CsCl Density Gradient Centrifugation of 1·707 g/cm^3 and 1·721 g/cm^3 Satellite DNA Preparations

The 1·707 g/cm^3 satellite DNA preparation is identical with fraction B_6 of Scheme 2, the 1·721 g/cm^3 satellite DNA preparation was obtained from fraction A_3 of Scheme 1 by precipitation with histone Fl. *a* 1·721 g/cm^3 satellite DNA, native; *b* 1·721 g/cm^3 satellite DNA, melted and reannealed to $C_0t = 0{\cdot}5$ mol s l^{-1} in 0·23M-Na$^+$; *c* 1·707 g/cm^3 satellite DNA, native; *d* 1·707 g/cm^3 satellite DNA, melted and reannelaed to $C_0t = 0{\cdot}5$ mol s l^{-1} in 0·23M-Na$^+$.

The satellite component of a density 1·721 g/cm^3 was isolated by using the advantage of the ability of histone F1 to bind into an insoluble complex preferentially the DNA with a lower content of G + C so that DNA richer in G + C remains free in solution[6]. As starting material we used fraction A_3 containing about 50% of the 1·721 g/cm^3 component. The precipitation procedure resulted in preparations enriched with this satellite DNA by about 80%, as follows from the CsCl density gradient profiles (Fig. 2*a*).



SCHEME 2

Scheme 2 shows the modified fractionation procedure using partial denaturation at 94°C. This procedure is more complicated but it allows to obtain besides both heavy satellites another defined satellite component of lower density (1·707 g/cm^3). This component is present in the fractions obtained after partial denaturation at 96°C only in a small amount and in a mixture with the heavier components. Both fractions A_6 and B_6, (Scheme 2), contain the 1·707 g/cm^3 component, fraction A_6 in mixture with the 1·714 g/cm^3 component, fraction B_6 in a relatively homogeneous form. Analysis of fractions A_7, B_7, C_7, B_5 and C_5 (not shown) indicates that these fractions are, similarly to most fractions shown in Fig. 1, mixtures of components of densities 1·714 and 1·721 g/cm^3. Fraction C_5 represents a practically homogeneous 1·714 g/cm^3 component. Fraction B_7 can be used for isolating the 1·721 g/cm^3 component using precipitation with histone F1. The A_7 fraction represents a mixture of low-molecular DNA fractions. The yields of the pure satellite components are in this case poorer than in the fractionation using heating to 96°C.

Properties of Isolated Satellite Components

The physicochemical parameters of preparations of satellite components homogeneous in density are shown in Table I. The density values of both heavier satellites (1·714 and 1·721 g/cm^3) as well as their T_m values (determined in standard saline citrate) agree satisfactorily with data in the literature[14]. The 1·707 g/cm^3 satellite component separated from the main DNA band on the basis of composition differences has not yet been described. Components of a similar density isolated in Cs_2SO_4–$-Ag^+$ gradients[15,16] may represent different sequences as will be shown later. The single-strand molecular weights of the isolated satellite components are relatively low perhaps due to single-strand breaks occurring on heating, but they exhibit no systematic trend.

TABLE I

Some Physicochemical Constants of Satellite DNA Preparations

The molecular weights were determined in the single-stranded state; values of T_m were measured (*a*) in standard saline citrate, (*b*) in 0·25 × standard saline citrate.

Sat. DNA ϱ, g/cm^3	Preparation	$s^{pH13}_{20,w}$	$M \cdot 10^{-5}$	T_m, °C	
				a	*b*
1·707	1	8·4	3·2	91·0	81·7
1·714	1	8·7	3·0	94·4	
1·714	2	13·9	11·2		85·3
1·721	1	7·0	2·0	96·7	88·6

The melting curves of all the three satellite components are shown in Fig. 3. The curve of the 1·707 g/cm^3 component is less steep than the curves of the other two components. In spite of their homogeneity in CsCl, the 1·714 and 1·721 g/cm^3 components are not homogeneous as to the melting sequences. It will be shown below that the low-melting sequences could be separated by thermal chromatography on hydroxyapatite. The gentle slope of the melting curve of the 1·707 g/cm^3 component also reflects a certain sequence nonhomogeneity.

Other differences between the satellite components follow on comparing the CsCl density gradient centrifugation profiles of native and melted and reannealed components. The density of the annealed DNA components 1·714 (Fig. 6) and 1·721 g/cm^3 (Fig. 2*b*) is almost identical with the density in the native state (Fig. 1*d* and 2*a*) while the 1·707 g/cm^3 component shows after annealing a density higher by some 7 to 8 mg/cm^3 (Fig. 2*c*, *d*). Incidentally, the densities of both reannealed lighter satellite components are identical which prevents their distinction after melting and reannealing. The sequence nonhomogeneity of all the three components follows also from the profiles of CsCl density gradient centrifugation of the reannealed components. In all cases there was found a band or shoulder corresponding to DNA of higher density

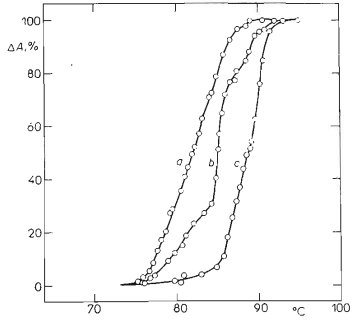


FIG. 3

Melting Curves of Isolated Preparations of Satellite Components

The curves were obtained at 260 nm in 0·25 standard saline citrate. *a* 1·707 g/cm^3 satellite DNA; *b* 1·714 g/cm^3 satellite DNA; *c* 1·721 g/cm^3 satellite DNA.

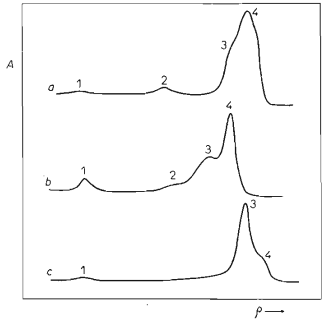


FIG. 4

Analytical Cs_2SO_4–Ag^+ Density Gradient Centrifugation of Isolated Preparations of Satellite Components

$r = 0{\cdot}35$, pH 9·2, ϱ $Cs_2SO_4 = 1{\cdot}5$ g/cm^3. *a* 1·707 g/cm^3 satellite DNA; *b* 1·714 g/cm^3 satellite DNA; *c* 1·721 g/cm^3 satellite DNA.

than that of most of the renatured DNA. This DNA may represent as much as 30–40% of the individual satellite components.

It follows from the results of some recently published papers[14–16] that isopycnic centrifugation in Cs_2SO_4–Ag^+ gradients is a powerful method for separating components with different, more or less homogenous sequences. In the present work we used an analytical modification of the method for attaining finer sequence characterization of the isolated satellite components. Fig. 4 shows the profiles of all the three preparations of satellite components in a Cs_2SO_4–Ag^+ gradient at pH 9·2 ($r = 0{\cdot}35$). The different components are designated in the individual diagrams with 1–4, in the order of increasing densities.

Thermal Chromatography of 1·714 g/cm³ *Satellite* DNA

Fig. 5 shows the elution diagram of thermal chromatography of melted and reannealed preparation ($C_0t = 0{\cdot}27$ mol . s . l^{-1} in 0·09M phosphate) of the 1·714 g/cm³ satellite component. The figure shows that the DNA is eluted in two peaks. The first peak is formed by fractions eluted at temperatures below 81°C, the second peak by fractions eluted at temperatures above 81°C. After annealing to $C_0t = 0{\cdot}5$ mol . s . l^{-1} in 0·23M-Na^+ the individual fractions were analyzed by CsCl gradient centrifugation and, according to the result, they were pooled into four larger fractions A, B, C and D (Table II). Fraction A contains DNA from the first peak and differs substantially from the other fractions. In a CsCl gradient (Fig. 6) it forms a single broad band at a density 1·722 g/cm³, *i.e.* by 8 mg/cm³ higher than the density of this satellite DNA in the native state. The melting curve of fraction A annealed to $C_0t = 0{\cdot}5$ mol . . s . l^{-1} in 0·23M-Na^+, corresponds to the melting curve of denatured DNA. The T_m is very low, 53°C in standard saline citrate. These results indicate that fraction A, representing almost 40% of the given sample of 1·714 g/cm³ satellite DNA contains DNA which cannot be renatured under the present experimental conditions. Fractions

TABLE II

Fractionation of the 1·714 g/cm³ Satellite DNA by Thermal Chromatography on Hydroxyapatite in 0·08M Phosphate ($NaH_2PO_4 + Na_2HPO_4$. 1 : 1)

Values of molecular weights were determined in the single-stranded state.

Fraction	Elution °C	DNA eluted %	$s^{pH13}_{20,w}$	$M . 10^{-5}$
A	up to 80·9	38	9·0	3·8
B	80·9–87·2	19	8·9	3·7
C	87·2–89·6	22	8·6	3·4
D	89·6–97·2	21	8·0	2·8

B, C and D all contain renatured satellite DNA banding at a density of 1·714 g/cm^3; fractions C and D also contain a renatured satellite DNA banding at a density of 1·721 g/cm^3 (Fig. 6). Bands of renatured satellite components are very sharp which indicates a high molecular weight.

DISCUSSION

The definition of the satellite component of DNA as used here follows the original definition by Kit[24] which consists in a separation of DNA in a CsCl density gradient. This definition is certainly valid for the components of densities 1·714 and 1·721 g/cm^3. The 1·707 g/cm^3 component is a cryptic satellite, *i.e.* in a CsCl gradient of unfractionated DNA it is not sufficiently distinguished from the main band. However, it could be separated by chromatographic methods in a defined form using the difference in its composition from the average composition of calf thymus DNA. Since the basis of separation in a CsCl density gradient of native DNA components consists above all in differences in the average G + C content, we are dealing here in both cases with components defined by their average composition. Another definition of the satellite component[1] is based on the separation of components in a Cs_2SO_4–Ag^+ (or Cs_2SO_4–Hg^{2+}) gradient which may be assumed to be sequence-specific. This definition is more stringent but it requires such knowledge about the character of the components as is not always available.

The methods most often used for isolation of satellite DNA components are those of preparative centrifugation in CsCl or $CsSO_4$–Ag^+ (-Hg^{2+}) gradients. In spite

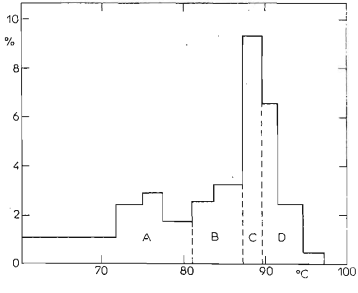


FIG. 5

Thermal Chromatography of Renatured 1·714 g/cm^3 Satellite DNA on Hydroxyapatite in 0·08M Phosphate (NaH_2PO_4 + Na_2HPO_4 1 : 1)

Fractions A–D are characterized in Table II.

of the advantages, in particular as to the relative purity of the components obtained, they have obvious disadvantages in being demanding for material as well as centrifuge time and are not suited for work with larger amounts of DNA. Of other methods the most suitable one for the isolation of native satellite components appers to be fractionation on methylated albumin – kieselguhr columns. When using this method for isolating the calf satellite DNA one should make use of the fact that calf satellite DNA's contain higher average amounts of G + C than the main-band DNA. The whole fractionation procedure may then be divided into two parts, the first dealing with a separation of the mixture of satellite DNA's from the main-band DNA, and the second with the separation of this mixture into the individual components.

The first part of the fractionation procedure was carried out by taking the advantage of the ability of the methylated albumin – kieselguhr columns to separate according to the G + C content as well as according to the secondary structure. Fractionation based on partial denaturation of DNA under conditions when mostly the main-band DNA is denatured and on the subsequent separation of the native fraction containing satellite DNA is very efficient and makes it possible to regulate the composition of the native fraction by selecting the denaturation temperature. It should be emphasized that the satellite components isolated here represent native DNA which has never been irreversibly denatured and that thus we are not dealing here with a preparation analogous to the isolation of repetitive DNA components on hydroxyapatite[25,26].

Separation of a mixture of satellite DNA's was done by the method of repeated fractionation on methylated albumin – kieselguhr columns. The results depend on the content of the given component in the mixture and on the G + C content diffe-

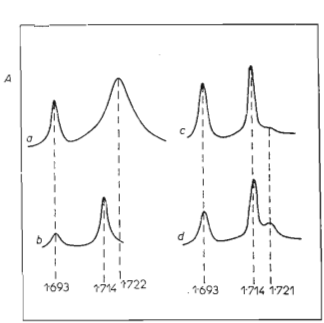


FIG. 6

CsCl Density Gradient Centrifugation of 1·714 g/cm³ Satellite DNA Fractions Obtained by Thermal Chromatography on Hydroxyapatite

All the fractions were reannealed to $C_0t = 0{\cdot}5$ mol s l^{-1} at 70°C in 0·23M-Na$^+$. *a* Fraction A; *b* fraction B; *c* fraction C; *d* fraction D.

rences exhibited by the individual components. The simplest of all is the isolation of the 1·714 g/cm^3 satellite DNA. The total yield is better in the case when the fraction obtained with the aid of partial denaturation at 96°C is used as the starting material. On the other hand, the 1·707 g/cm^3 satellite DNA component can be isolated only starting with the fraction obtained by partial denaturation at 94°C. Isolation of homogeneous 1·721 g/cm^3 satellite DNA by fractionation on methylated albumin – kieselguhr columns was not possible, apparently because of the polydispersity of the molecular weights which results partly in the simultaneous elution of both heavy satellite components. The isolation method based on the interaction with histone F1 (ref.[6]) was found to be satisfactory for the present purpose.

Although the density in a CsCl gradient permits to distinguish only three satellite components of densities of 1·707, 1·714 and 1·721 g/cm^3 the melting curves and CsCl density gradient profiles of melted and reannealed preparations indicate that the components are heterogeneous in their sequences. The diagrams of reannealed components permit to assess that in all the cases studied here some 30–40% is renatured under the same annealing conditions up to a much lower degree than the remainder of the preparation. In the case of the 1·714 g/cm^3 satellite DNA we could separate the two components of reannealed DNA by thermal chromatography on hydroxyapatite (Fig. 5).

A reason for the presence of components renaturing to different degree in the density-homogeneous preparations of satellites might lie in the presence of partly denatured moleculess with an originally lower native density. However the high density values of the non-renaturing components in the reannealed state (1·722 to 1·731 g/cm^3) exclude the possibility that we might be dealing here with a partly denatured DNA of the main band, the densities of which after melting and reannealing lie between 1·709 and 1·715 g/cm^3 (ref.[16,21,27,28]). It thus appears that we have to assume sequence heterogeneity within the individual density-homogeneous preparations of satellite components or, according to the definition of Walker[1], the presence of several satellite components with defined sequence in density-homogeneous preparations.

We attempted to obtain further information on the components with defined sequence in our satellites by an analysis in Cs_2SO_4–Ag^+ gradients. It is rather difficult to identify the individual components with defined sequence with the components found by Filipski and coworkers[16], because the density of a number of components in a Cs_2SO_4–Ag^+ gradient depends steeply on the binding ratio Ag^+/nucleotide. Since the effective value of this ratio for the individual components can differ from case to case due to the presence of other components we relied on the sequence of densities in the Cs_2SO_4–Ag^+ gradients rather than on the absolute density values. For better clarity we shall designate the components isolated on the basis of separation in a preparative Cs_2SO_4–Ag^+ gradient and defined by densities in a CsCl gradient (ref.[16]) with subscript Ag.

It follows from Fig. 4*a* that the preparation of 1·707 g/cm^3 satellite DNA is sepa-

rated in the Cs_2SO_4–Ag^+ mainly into two components 3 and 4 with similar densities. The main component 4 was identified with the component $(1{\cdot}704\ g/cm^3)_{Ag}$. This assignement is supported by the agreement in the densities of the reannealed components $1{\cdot}707\ g/cm^3$ (Fig. 2*d*) and $(1{\cdot}704\ g/cm^3)_{Ag}$ (ref.[16]) and further by the broad melting curve found in both cases. It is likely that the other major component 3 can be identified with component $(1{\cdot}709\ g/cm^3)_{Ag}$, (ref.[16]) characterized also by considerable intramolecular heterogeneity. The resulting value of density, $1{\cdot}707\ g/cm^3$, agrees well with the fact that we are dealing mainly with a mixture of these two rather similar components.

The major component 4 of the preparation of $1{\cdot}714\ g/cm^3$ satellite DNA (Fig. 4*b*) is undoubtedly identical with the repetitive sharply melting component $(1{\cdot}714\ g/cm^3)_{Ag}$ (ref.[16]). The nature of the second, poorly renaturing component with a lower melting temperature would indicate it to correspond to $(1{\cdot}709\ g/cm^3)_{Ag}$ (ref.[16]). However, the density of this melted and reannealed component is $1{\cdot}722\ g/cm^3$ and hence higher than that reported by Filipski and coworkers[16]. The preparation of the $1{\cdot}721\ g/cm^3$ satellite DNA (Fig. 4*c*) contains the two sharply melting repetitive components $(1{\cdot}723\ g/cm^3)_{Ag}$ and $(1{\cdot}714\ g/cm^3)_{Ag}$ (peaks *3* and *4*). The third component with density after melting and reannealing of $1{\cdot}731\ g/cm^3$ (Fig. 2*b*) does not correspond to any of the components reported by Filipski and coworkers[16].

The isolated satellite preparations contain further small amounts of other components with much lower densities in the Cs_2SO_4–Ag^+ gradient (1 and 2). By their densities they might be identical with $(1{\cdot}710\ g/cm^3)_{Ag}$ and $(1{\cdot}719\ g/cm^3)_{Ag}$ of Filipski and coworkers[16].

It is of interest that our preparations of the satellite DNA components might be found to contain all the important components of densities above $1{\cdot}704\ g/cm^3$ described by Filipski and coworkers[16], with the exception of the sharply melting component $(1{\cdot}705\ g/cm^3)_{Ag}$. This component which may be readily isolated and detected in the $CsSO_4$–Ag^+ gradient as a very light component[15,16] is present in the preparation of the $1{\cdot}707\ g/cm^3$ satellite DNA at most in traces (component 1, Fig.4*a*) and is likely to have been denatured during the partial denaturation in the course of preparation. The non-renaturing components of the present satellites are probably intramolecularly heterogeneous sequences of DNA containing segments with high G + C content which render these molecules resistant to irreversible denaturation and endue them with a high density in the CsCl gradients. It is also possible that these sequences are in the native state at least partly bound to the repetitive satellite sequences and that they are released only after complete denaturation of the preparation. As follows from the value of single-strand molecular weights (Table I) substantial degradation of molecules takes place (the mean molecular weight in the native state is $3-7 \cdot 10^6$ (ref.[12])). This is obviously due to the great number of single-strand breaks caused by heating during the isolation procedure. On the basis of the present results it is difficult to decide whether we are dealing with mere coincidence in density or

whether the heterogeneous sequences are structurally associated with the repetitive satellite sequences, *e.g.* as spacers[29].

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