ISOLATION OF A HIGHLY REASSOCIATING FRACTION OF CHINESE CABBAGE NUCLEAR DNA AND ITS ROLE IN RIBOSOMAL RNA SYNTHESIS

by

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ABSTRACT

Nuclear DNA of Chinese Cabbage leaf tissue contains a major DNA component plus a satellite component. The buoyant density of the satellite in CsCl was determined by analytical density gradient centrifugation to be 1.706 g/cc. This satellite was isolated in partially purified form and tested for hybridization with rRNA. Satellite DNA was found to be rich in rRNA cistrons as compared to the remaining or main band DNA.

When denatured and incubated at conditions favorable for renaturation, a portion of the satellite DNA returns to a double helical state approximating its native structure, while the main band DNA reassociates in the form of intermolecular networks. The network particles occupy a narrow band in a CsCl gradient and denature over a temperature range of 40°C. The renaturable fraction is believed to be composed entirely of satellite DNA.

Satellite DNA was estimated to be composed of from 46.9% to 50.5% guanine + cytosine (G + C) content, and to contain a substantial quantity of odd bases. In contrast, main band DNA was estimated to contain from 40.8% to 43.2% G + C and a smaller proportion of odd bases.

It may be possible to isolate a purified renatured satellite which would be suitable for further investigation.

INTRODUCTION

When centrifuged to equilibrium in a density gradient, DNA is distributed at a position in the gradient corresponding to its own buoyant density. Equilibrium centrifugation of the nuclear DNA of Chinese Cabbage revealed the presence of a major and a minor DNA component, which, owing to their differences in buoyant density, were distributed at different positions in the gradient. Such a minor component has been referred to as a "satellite."

That the nature of the two respective components gave rise to unequal buoyant densities in the CsCl gradient suggested the possibility that the satellite DNA was a unique fraction of DNA, perhaps differing in other ways from that of the major component.

It was undertaken to isolate the DNA comprising the satellite component and characterize it physically and functionally in an effort to define its role in cellular processes.

LITERATURE REVIEW

Isolation and Characterization of DNA

Although a variety of methods have been employed for the fractionation of DNA from tissue of single species of organisms (Bendich, Fresco, Rosenkranz, and Beiser, 1958; Bendich, Russel, and Brown, 1953; Walker and McClaren, 1965), equilibrium density gradient centrifugation has been most often used for the detection and fractionation of multi components of DNA.

The technique of centrifugation of DNA in a density gradient of CsCl was originally defined by Meselson, Stahl, and Vinograd (1957), who showed that the equilibrium position of DNA in a density gradient was determined by the buoyant density (ρ) of the DNA. A means of fractionation of DNA on the basis of buoyant density was thus afforded. A greater resolution and increased capacity has been achieved by the use of the fixed angle rotor for equilibrium density gradient centrifugation (Flamm, Bond, and Burr, 1966).

Utilization of "isopycnic centrifugation" established that a linear relationship existed between buoyant density and the quanine + cytosine (G + C) content of DNA. (Sueoka, 1959; Marmur and Doty, 1959; Rolfe and Meselson, 1959). G + C content of DNA was found also to be directly proportional to the thermal denaturation temperature (Tm) (Marmur and Doty, 1959; Doty, Marmur, and Sueoka, 1958). Continuing investigations of the dependence of Tm and ρ on base composition of

DNA from a variety of organisms (Marmur and Doty, 1962; Schildkraut, Marmur, and Doty, 1962; Sueoka, 1961) further defined these phenomena. Noteworthy is the inapplicability of these relationships for DNA containing substantial quantities of bases other than quanine, cytosine, thymine, and adenine. Numerous cases have been reported where inconsistent values of G + C content have been derived from Tm and ρ (see for example: Matsuda and Siegel, 1967; Greenberg and Uhr, 1967; Flamm, Bond, Burr, and Bond, 1966). The presence of methylated bases and bases other than the four common ones may severely alter the thermal and hydrodynamic properties of DNA (Schildkraut, Marmur, and Doty, 1962; Marmur and Doty, 1962; Kirk, 1967). The ubiquitous heterogeneity, the presence of minor fractions of organelle origin, and the occurrence of odd bases in the DNA from higher plants necessitate a cautious interpretation of data obtained from thermal denaturation and density gradient centrifugation. These methods, however, have often been useful for the characterization of DNA.

The Presence of Satellite DNA

Numerous satellites have been reported for whole tissue DNA. Many of these have been shown to originate in subcellular organelles. Attention is directed, in this thesis, to those satellites of nuclear origin. Nuclear satellite DNA has been found for mammals (Borst and Ruttenberg, 1966; Flamm, Bond, Burr, and Bond, 1966), and a variety of species of higher plants (Matsuda and Siegel, 1967). Matsuda and Siegel have shown by hybridization studies (Gillespie and Spiegelman, 1965)

that a biochemical role of plant satellite DNA may be the synthesis of rRNA (ribosomal RNA). By heterologous RNA-DNA hybridization they have also shown that rRNA of different plant species is remarkably similar in nucleotide sequence. This is the basis upon which P^{32} tobacco ribosomal RNA was used to test hybridization with Chinese Cabbage DNA as reported herein.

Intracellular Localization and Function

The site of cellular rRNA synthesis is believed to be the nucleolus and the DNA associated with nucleoli has been postulated to function in the synthesis of rRNA (for reviews, see Perry, 1967; Birnstiel, 1967). Firm evidence exists for the assignment of satellite DNA to the nucleolus. The amount of DNA complementary to rRNA in anucleolate mutants of Drosophila (Ritossa and Spiegelman, 1965) and Xenopus (Wallace and Birnstiel, 1966) is diminished as compared to that of the wild type with the normal complement of nucleoli. Ambiguities presently exist in the results of direct characterization of nucleolar DNA. rRNA cistrons were, however, considerably enriched in an isolated nucleolar DNA fraction from Hela cells (McConkey and Hopkins, 1964). It is possible that satellite DNA may be chromosomal or extrachromosomal, and may be located at the nucleolar organizer (see above three references), in nucleoli free of the chromosomes (Miller, 1964) or randomly distributed throughout the genome (Bond, Flamm, Burr, and Bond, 1967).

METHODS AND MATERIALS

DNA Extraction

DNA was extracted from the leaves of two-four week old Chinese Cabbage (Brassica Pekinensis Lour. Rupr. Van. Wongbok) plants. Approximately 1000 grams of de-ribbed Chinese Cabbage leaves was first ground in an ordinary meat grinder and collected in a container of sucrose-tris buffer (0.5M sucrose, 0.01M $MgCl_2$, 0.05M tris, 0.025M KCL, 0.005M mercaptoethanol, pH 8.2) (Marcus and Feeley, 1964). This was immediately transferred to a chilled mortar and pestle and ground with sand. Approximately 100 ml grinding buffer was used for every 250 grams of leaf tissue. The homogenate was filtered through two layers of cheesecloth and glasswool and centrifuged at 3000 rpm for 5 minutes at 1°C in the Sorvall RC-2. Pellets were twice resuspended in, and washed with, about 25 ml sucrose-tris buffer with 3.5% triton-X added. The nuclei were collected after each treatment by centrifugation. Nuclear pellets were then defatted by homogenation of a suspension of pellets in each of the following in succession: 1. 95% EtOH, 2. EtOH: Ether (1:1, V/V), 3. Ether, 4. Petroleum ether, 5. CCl_4 cyclohexane (1:4, V/V), 6. Ether, 7. EtOH: Ether (1:1, V/V), 8. EtOH (Hotta and Bassel, 1965). Variable amounts of each reagent were used, depending upon the size of the pellets. Each pellet was then suspended in 20 ml BPES buffer (0.006M Na_2HPO_4, 0.002M NaH_3PO_4, 0.001M disodium EDTA, 0.179M NaCl, pH 9.0) Crothers and Zimm, 1965), to which 0.3M 4-amino-salicylic acid and 1% SDS had previously been added. Suspensions were kept on ice until

viscous. Twenty ml phenol (to which 0.1% 8-hydroxyquinoline had been added and which had been subsequently washed twice with equal volumes of 0.1 BPES buffer) was then combined with the suspension of pellets and mechanically shaken. The duration of the phenol treatment was increased for more effective deproteination (Yolles and Freeman, 1967) which may have resulted in a decrease in the yield of satellite DNA (Skinner and Triplett, 1967) obtained in some experiments. Layers were then separated by centrifugation for 5 min. at 5000 rpm and the buffer layers carefully removed and combined. DNA was precipitated by the addition of two volumes of 95% EtOH. DNA was purified by two treatments with chloroform to remove protein and an intervening incubation with RNase. Further purification steps are as given by Marmur (1961).

Hybridization

Hybridization of total and fractionated Chinese Cabbage DNA to tobacco ribosomal RNA was as described by Gillespie and Spiegelman (1965). Fifty µg samples of total DNA and 10 µg samples of satellite and main band DNA were denatured by heating at 98°C for 10 min., quick-cooled in ice, and imbedded on B-6 Bac-T-flex membrane filters (Schleicher and Schuell Co.). Ten µg and 20 µg of P^{32} labelled tobacco leaf ribosomal RNA, with a specific activity of 4629 cpm/µg, were incubated with each of the DNA samples for 8 hours at 68°C in 5 ml 2XSSC (1XSSC = .15M NaCl, 0.015M trisodium citrate, pH 7.0). Ten µg RNA was found to be sufficient for saturation of DNA. Final cpm values were corrected for background (20 cpm) and radioactivity arising from nonspecific

retention of RNA on the membranes (14 cpm). P^{32} emissions were counted with a Nuclear Chicago thin window gas-flow counter.

Preparative Centrifugation and Fractionation

Preparative fixed angle centrifugation was accomplished using the Spinco model L-2 with the no. 50 rotor (Flamm, Bond, and Burr, 1966). Variable amounts of DNA (200-300 ug) was added to Beckman Polyallomer tubes containing a 62% aqueous CsCl solution (w/w), and the density adjusted to 1.708 g/cc (± .006) by the addition of H_2O. Alternately solid CsCl was added to the appropriate volume of dilute DNA solution. Density was determined refractometrically with a Zeiss refractometer (Ifft, Voet, and Vinograd, 1961). Centrifugation and refractometry were conducted at 20°C. After centrifugation, tubes were fitted to a fraction collection apparatus and a 20 G needle was inserted into the bottom of the tube. Twelve drop fractions were collected and 1 ml of 0.1XSSC added to each. The O.D. at 260 mμ was determined with a Gilford model 240 spectrophotometer. Desired fractions were pooled and the DNA precipitated by the addition of 2 volumes 95% EtOH.

Analytical Centrifugation

Analytical isopycnic centrifugation was performed with the Spinco Model E analytical ultracentrifuge equipped with ultraviolet optics. Three μg to 5 μg DNA, along with 1 μg marker DNA, was added to a CsCl solution and the final density adjusted to 1.708 g/cc (± .006) in the manner described for preparative centrifugation. Marker DNA was from Micrococcus lysodeikticus (ρ =1.731) (Schildkraut, Marmur, and Doty,

1962). Cells were packed with the single sector Kel F centerpieces. Centrifugation was with the AN-F rotor at 44,000 rpm for 18 hours at 20°C. Ultraviolet absorption photographs were traced with the Spinco model RB analytrol. The buoyant density of DNA was determined by the method of Sueoka (1961). The buoyant density of a given sample of DNA is expressed by the relationship:

$$\rho = \rho_o + 4.2\omega^2(r^2 - r_o^2) \times 10^{-10} \text{ g/cc} \qquad (1)$$

where ρ_o is the buoyant density of marker DNA, and r_o and r are the distances from the axis of rotation of the band formed at equilibrium by marker DNA and DNA of unknown density. At 44,000 rpm, it can be shown that:

$$\rho = \rho_o + 0.00892\ (r^2 - r_o^2) \text{ g/cc} . \qquad (2)$$

Appropriate measurements from the densitometer tracings, along with knowledge of actual rotor distances, permit calculation of mean buoyant densities by equation 2.

Thermal Denaturation

Investigation of the thermal properties of DNA was as established (Doty, Marmur, and Sueoka, 1958; Marmur and Doty, 1962). Thermal transition profiles of DNA were obtained by simultaneously recording the temperature and absorbancy at 260 mμ of DNA solutions with the Gilford 2000 recording spectrophotometer. Temperature gradients were generated (Haacke temp. circulator) about 1 cm closed cuvettes. The rate of

temperature increase usually employed was 0.25°C per min. Alternately, temperatures were observed from a calibrated thermometer inserted in the cuvette chamber. The degree of compositional heterogeneity of DNA samples was estimated from the temperature interval over which the denaturation process was from 16% to 84% completed (Franklin, 1967).

Denaturation and Reassociation

Native DNA was denatured by heating at 98°C for 10 min. in 0.1XSSC. A sufficient quantity of concurrently heated concentrated salt solution (10 X SSC) was added to the solution of DNA at the end of the denaturation period to attain the final desired salt concentration. Slow cooling to 68°C ($\pm$ 1) was followed by incubation at this temperature for 8 hours.

RESULTS

When nuclear DNA of Chinese Cabbage leaf tissue is subjected to preparative isopycnic centrifugation, a distinct skewness is observed in the density profile of the banded DNA (Fig. 1). The same result was previously obtained by Matsuda and Siegel (1967). There is a fraction of DNA (A) with a distinctly higher mean buoyant density than the remaining or main band DNA (B). The estimated mean buoyant density of the respective components is 1.706 g/cc and 1.699 g/cc. The more dense fraction (A) constitutes approximately 7% of the total nuclear DNA. A third component (C) present in very small quantities, and of considerably higher buoyant density, is also revealed. The origin and nature of this component is unknown. Profiles similar to that of Fig. 1 were obtained, after centrifugation in CsCl, with each extract of DNA from mature leaf tissue.

Analytical isopycnic centrifugation of an aliquot of nuclear DNA resulted in a similar distribution as seen in Fig. 2 (a). Fractionation of DNA by preparative centrifugation in CsCl was undertaken in an attempt to isolate the satellite DNA. Analytical centrifugation of the pooled fractions of shaded area "A" of Fig. 1 revealed that the selected portion was greatly enriched in satellite but probably contained some main band DNA - see Fig. 2 (b). However, due to the small quantity of satellite DNA and the nearness of its buoyant density to that of the main band DNA, experimentally useful quantities of pure satellite DNA

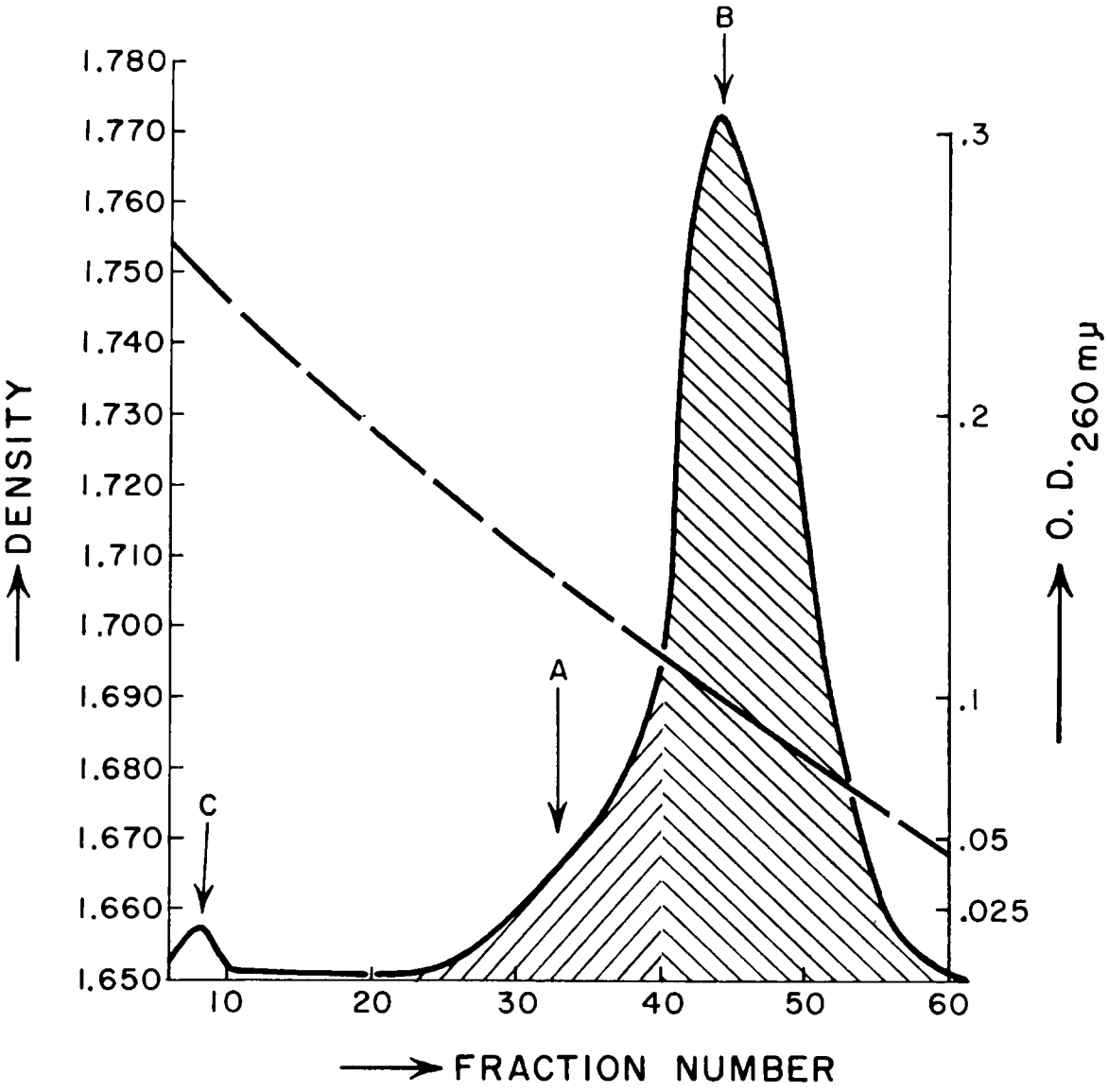

Figure 1. Profile of Chinese Cabbage DNA After Angle Head (Spinco No. 50) Centrifugation in CsCl at 35,000 rpm for 70 Hours at 20°C.

After centrifugation, a 20 g needle was inserted into the bottom of the tube and 12 drop fractions were collected. The O.D. at 260 mu of each fraction was determined after addition of 1 ml 0.1XSSC. The density of every fifth fraction was determined by refractometry immediately as it was collected. ———, $O.D._{260m\mu}$; ------, Density.

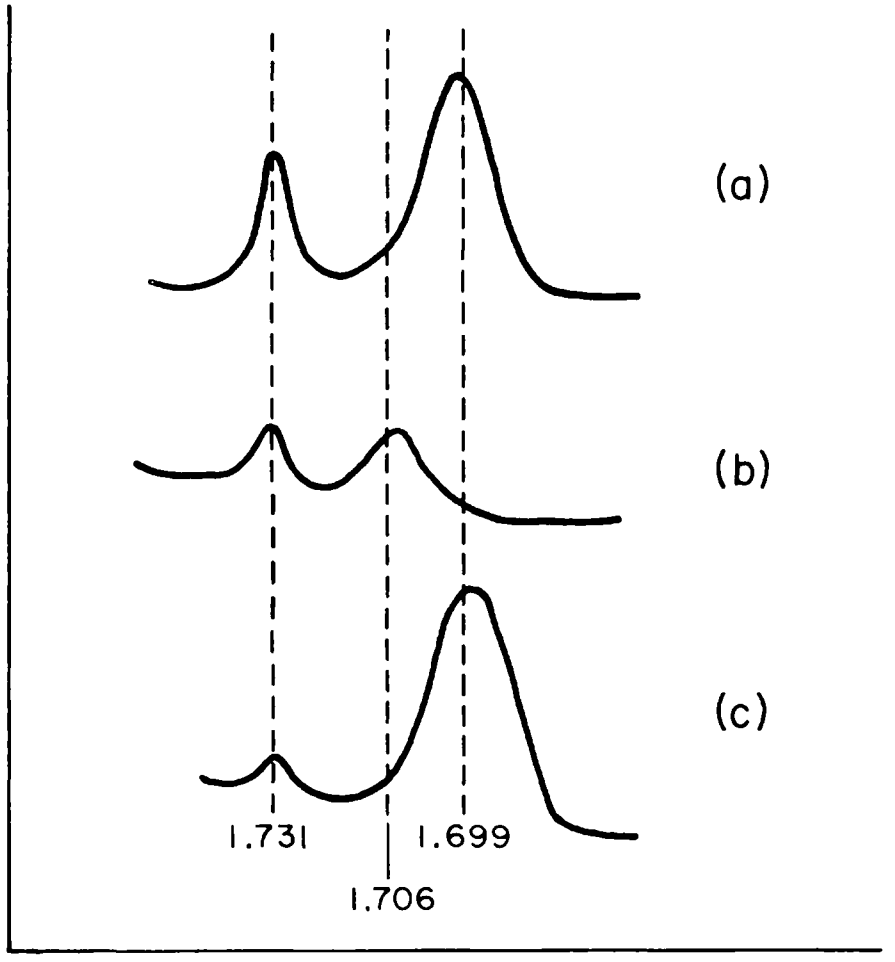

Figure 2. Analytical Centrifugation of Total and Fractionated DNA.

Densitometer tracings of U. V. photographs of (a) total nuclear DNA, (b) enriched satellite, (c) purified main band DNA. Photographs were taken after 18 hour centrifugation at 44 K rpm at 20°C in a Spinco model E analytical ultracentrifuge.

could not be obtained by two cycles of centrifugation of the native DNA. Analytical centrifugation of fractions comprising shaded area "B" of Fig. 1 revealed that main band DNA could be successfully purified as shown in Fig. 2 (c).

It was decided to test a sample of DNA enriched in satellite for the amount of hybridization with rRNA in order to determine if satellite DNA might contain a disproportionately large number of rRNA cistrons compared to main band DNA. This was suspected from the results of Matsuda and Siegel (1967) that: (1) the presence of a pumpkin satellite, which was found to be rich in rRNA cistrons, accounted for the high hybridization of pumpkin DNA with rRNA, and (2) Chinese Cabbage DNA, which also contained a satellite component, hybridized to a high extent with rRNA compared to DNA of other plant species lacking such a satellite. The results are shown in Table 1.

It can be seen from Table 1 that a sample of DNA enriched to approximately 45% satellite DNA specifically formed twice the amount of DNA-rRNA artificial hybrid as did main band DNA. It is estimated that 3% of the pure satellite DNA would be complementary to rRNA. The degree of hybridization is taken as evidence that satellite DNA contains a large number of nucleotide sequences complementary to those of rRNA while the main band DNA contains, in comparison, a fewer number of such sequences. Substantiation of a reference for the experimental method is provided by the hybridization value of tobacco DNA (.087%) which is similar to values obtained by Matsuda and Siegel (1967) and Matsuda (unpublished results).

TABLE 1

HYBRIDIZATION OF TOTAL, ENRICHED SATELLITE, AND PURIFIED MAIN BAND DNA TO P^{32} LABELLED TOBACCO RIBOSOMAL RNA

DNA Fraction	Purity of Components	Replicate Number	µg DNA per Replicate	Net cpm	Average[1] Corrected cpm	Hybrid-ization[2] (%)
Total	--	1	50	2857		
		2	50	2299		
		3	50	2439	2545	1.1
		4	50	2703		
		5	50	2597		
Main Band	--	1	50	1770		
		2	50	1681		
		3	50	2062	1799	0.77
		4	50	1818		
Satellite	45	1	10	755		
		2	10	730		
		3	10	800		
		4	10	885	3769	1.62
		5	10	637		
		6	10	833		

Table 1.--Continued

DNA Fraction	Purity of Components	Replicate Number	µg DNA per Replicate	Net cpm	Average[1] Corrected cpm	Hybrid-ization[2] (%)
Tobacco	--	1	50	192		
		2	50	227		
		3	50	263	201	0.087
		4	50	255		

1. Final cpm values are corrected for nonspecific retention of RNA on membranes (14 cpm) and background (20 cpm). Odd number replicates were incubated with 10 µg rRNA and even numbered with 20 µg rRNA (4,629 cpm/µg). Sufficient saturation was achieved with 10 µg rRNA and values for each set of replicates averaged.

2. Hybridization values are expressed as % DNA complementary to rRNA.

Further evidence for the uniqueness of satellite DNA both in base composition and sequence was provided by a study of the reassociation properties of denatured satellite DNA. When a sample of total nuclear DNA is denatured by heat, quick cooled, and subjected to isopycnic centrifugation, the single stranded DNA is distributed about a point .013 g/cc higher than native main band DNA. If denatured DNA is incubated at 68°C, as described in Methods and Materials, in a solution of 2XSSC and centrifuged in the CsCl gradient, the DNA is distributed in a bimodal pattern, showing a component with a mean buoyant density slightly less than denatured DNA and another at the density of native satellite DNA. Matsuda and Siegel detected the presence of a renaturable fraction of Chinese Cabbage DNA (1967). Fig. 3 shows the results of analytical centrifugation of the DNA after each of these successive treatments.

A very similar observation has been described for reassociated mouse DNA by Waring and Britten (1966). These authors have attributed the presence of a sharp band at the density of denatured DNA as being due to imperfect reassociation between many single stranded main band DNA molecules with few regions of complementarity. Direct physical evidence for such a large intermolecular network has been presented for a renatured yeast nuclear and mitochondrial satellite DNA (Sinclair et al., 1967). That some base pairing interactions did occur between strands of Chinese Cabbage main band DNA is suggested by the appearance of the network band at a slightly lower density than the mean density

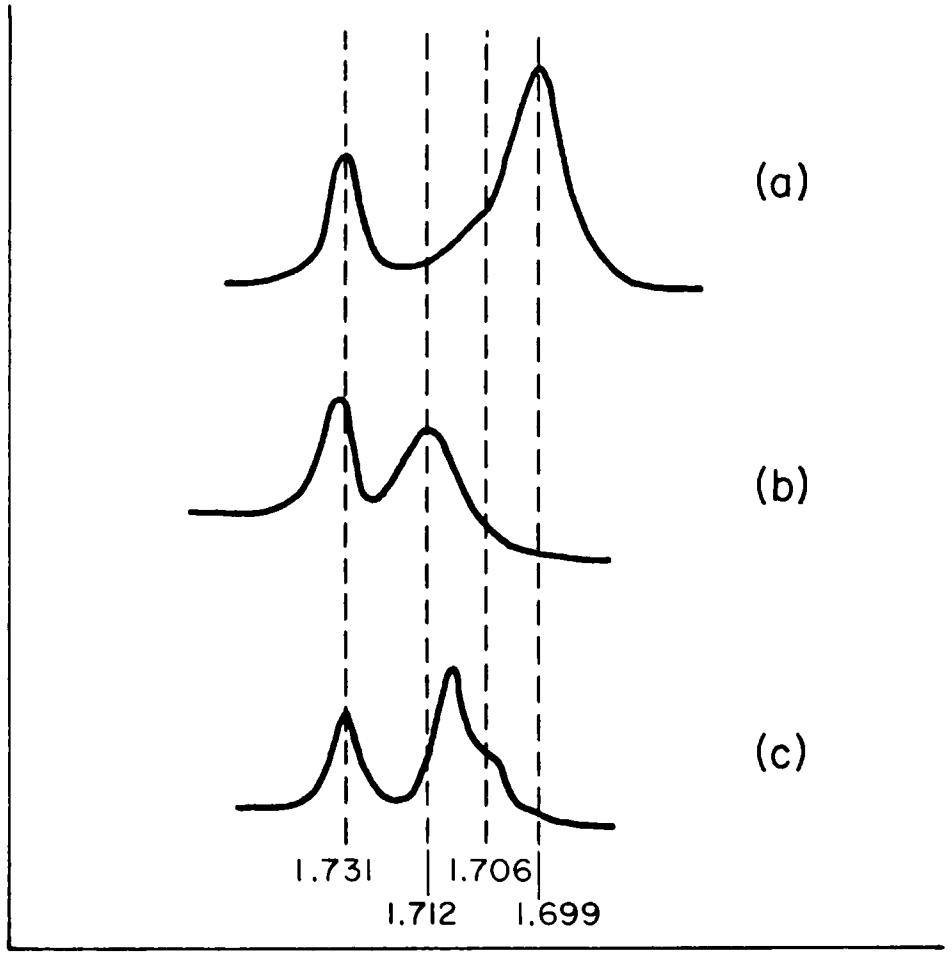

Figure 3. Analytical Centrifugation of Native and Thermally Treated DNA.

Densitometer traces of U. V. absorption photographs of (a) 3 µg native, (b) 3 µg heat denatured, c. 3 µg heat denatured DNA incubated in 2XSSC at 68°C for 8 hours. Conditions of centrifugation are as described in Fig. 2 and Methods and Materials.

of denatured DNA. It is possible that the higher melting satellite DNA (Tm = 90°C) was not fully denatured as seen in Fig. 3 (b).

From the distribution of reassociated DNA one might suspect that the minor, less dense component, consists of completely renatured satellite DNA. The peak density of this fraction is equal to that of the satellite enriched by angle head centrifugation shown in Fig. 2 (b).

It is evident that the satellite component consists largely of a fraction of DNA which is unique in its ability to renature to a state approximating its native double helical structure.

An attempt was made to isolate the renaturable component by preparative centrifugation. It was hoped that separation of the renaturable component from the more dense fractions of main band DNA could in this way be achieved. The resultant absorbancy profile of such an attempt, using only a small amount of DNA, is shown in Fig. 4.

It can be seen from Fig. 4 that the renatured satellite DNA (A) is partially resolved from the network particles of reassociated main band DNA (B) in a sample previously enriched in satellite. Collection and subsequent analysis of fractions comprising shaded area "A" of Fig. 4 revealed the presence of a small amount of component "B" present as an impurity in the fractions collected. An improved fractionation technique should permit the isolation of pure renatured satellite DNA. This accomplishment is significant not only as a means of routine isolation, but to allow further characterization of the seemingly completely renatured fraction of satellite. Further investigation of the physical

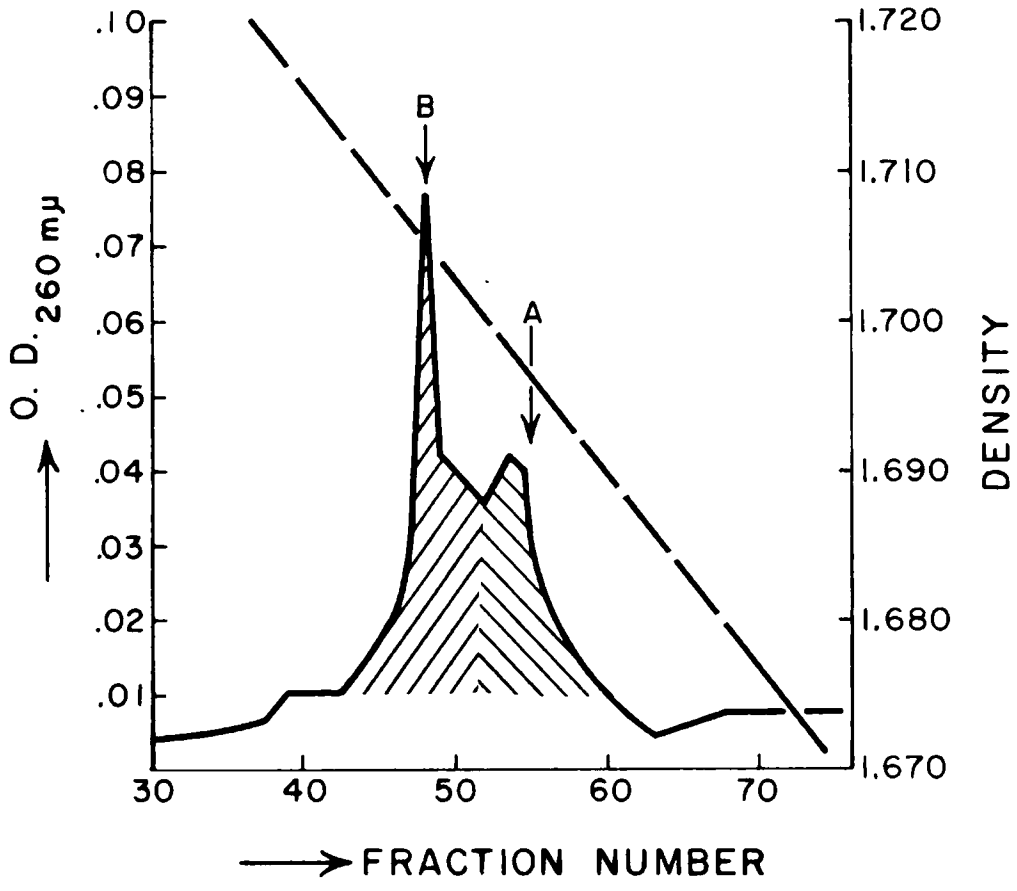

Figure 4. Absorbancy Profile of Reassociated DNA After Angle Head Centrifugation in CsCl.

DNA enriched in satellite was denatured by heating 10 minutes at 98°C and incubated for 8 hours at 68°C in 2XSSC. Conditions of centrifugation and fractionation are as described in Fig. 1 and Methods and Materials. ————, $O.D._{260\ mu}$; - - - - - -, Density.

properties of the DNA was undertaken. Thermal denaturation profiles of total nuclear DNA and fractionated components are shown in Fig. 5. Pertinent parameters are listed in Table 2.

TABLE 2

THERMAL DENATURATION CHARACTERISTICS
OF TOTAL AND FRACTIONATED DNA

	Tm	2σ	G + C#	G + C*
Total Nuclear	86°C	14	40.7%	40.8%
Main Band	87°C ($\pm$ 2)	8	43.2%	40.8%
Satellite	90°C	10	50.5%	46.9%

Details of the experimental conditions and methods of calculation are described in Methods and Materials. 2σ values are corrected for the natural transition (Δt = 3) of the homopolymer duplex dA:dT (Doty, Marmur, and Sueoka, 1958).

#. Values of % G + C calculated from Tm.

*. % G + C calculated from ρ.

Unfractionated DNA exhibits a broad transition profile (2σ = 14), reflecting considerable compositional heterogeneity. The contribution of satellite DNA is neglected in the calculation of G + C content of total DNA based upon ρ.

Considerable variation (85°C - 89°C) in Tm of samples of main band DNA was observed. Inclusion of the more dense fractions of the main band peak in a sample led to a higher Tm. Although the presence of small amounts of satellite in these fractions may be a contributing

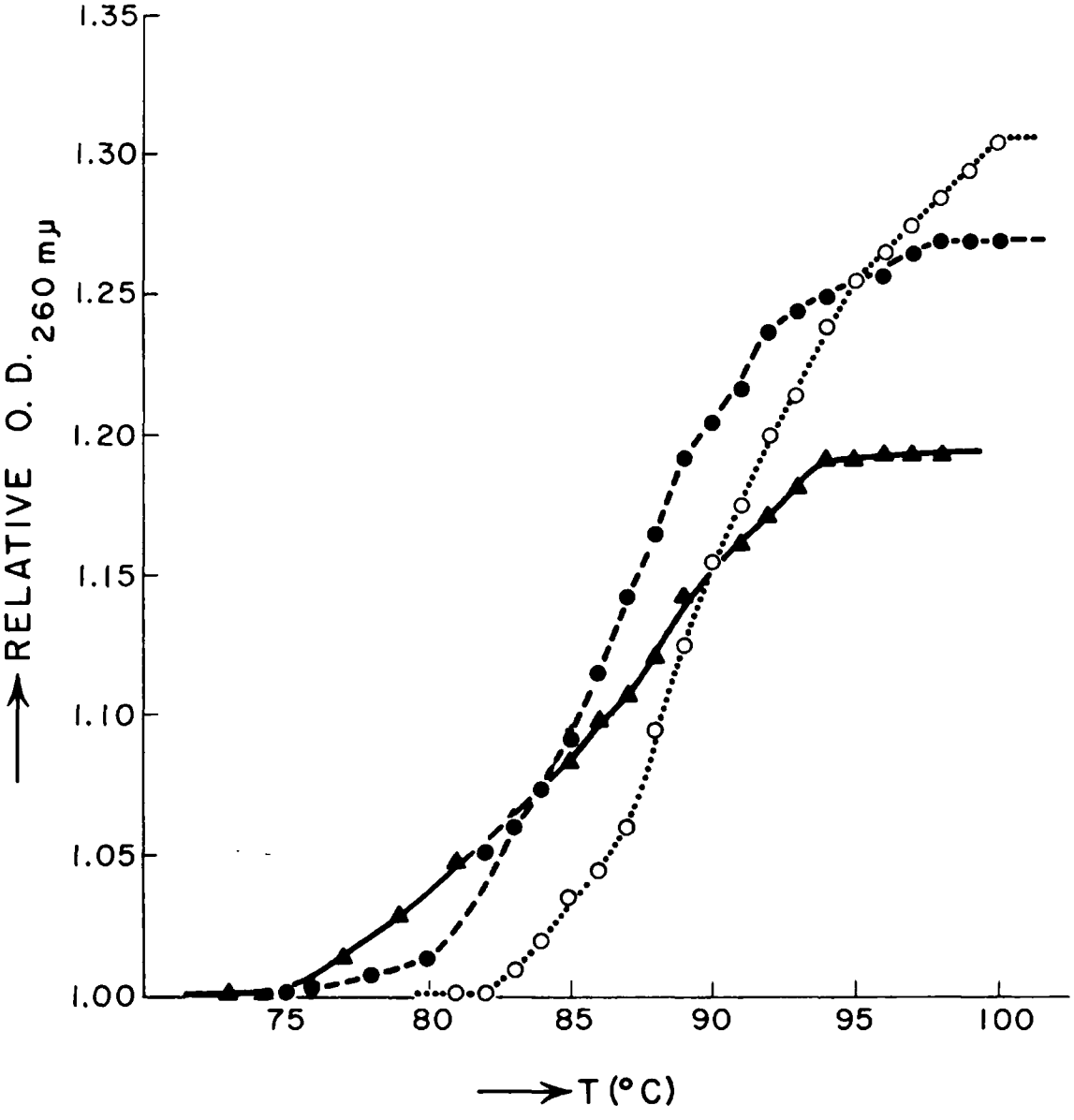

Fig. 5. Thermal Denaturation Profiles of Total Nuclear DNA and Fractionated Components.

Denaturation was followed by recording the $O.D._{260m\mu}$ at small temperature intervals over which the transition occurred. ▲-▲-▲, total nuclear; ●-●-● , purified main band; ○-○-○ , partially purified satellite.

factor, the variation is likely a reflection of heterogeneity within the main band as well. If the true Tm of purified main band DNA is equal to or very slightly less than that of total nuclear DNA, then the value of G + C content of main band (40.7%) derived from Tm (86°C) would be remarkably close to that (40.8%) derived from ρ (1.699 g/cc). This is in contrast to the corresponding dissimilar values calculated for satellite DNA (50.5% and 46.9%). This may indicate the presence of considerable numbers of odd bases in satellite DNA and a relative absence of such in main band DNA. Mouse satellite DNA (Bond, Flamm, Burr, and Bond, 1967) has been found to contain 5 methyl cytosine, and a difference between the estimate of base composition derived from Tm, and that derived from ρ, has been reported.

A considerably higher G + C content is observed for satellite than for main band DNA. It is expected that complete purification of the satellite would lead to a higher Tm and a further narrowing of the thermal transition profile.

Further evidence for the imperfect reassociation of main band DNA upon denaturation and incubation is indicated in Fig. 6. The profile more closely approximates that of a random coil than a double helix and occurs over a temperature range of 40°C. Single stranded regions of DNA are apparently present within the reassociated network particles.

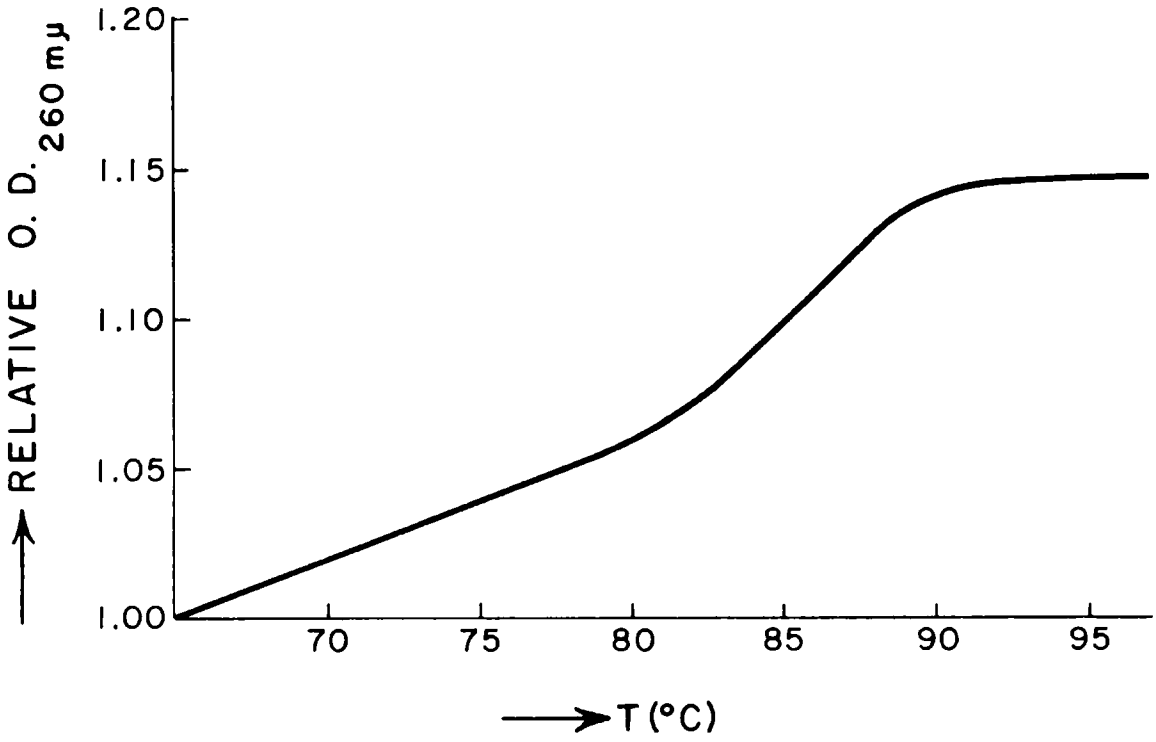

Figure 6. Thermal Transition Profile of Reassociated Main Band DNA.

Network particles were collected by preparative centrifugation of reassociated DNA (see text and Methods and Materials) and heated in 1XSSC. $O.D._{260m\mu}$ was determined as in Fig. 5.

DISCUSSION

The satellite of Chinese Cabbage nuclear DNA has been isolated in partially purified form and has been shown to contain a greater number of regions complementary to total tobacco leaf rRNA than does the main band DNA. Partial characterization of satellite DNA included estimation of base composition and an investigation of the process of renaturation of this component.

It is believed that the appearance of the satellite component in a CsCl gradient is due to the presence of a renaturable fraction of DNA which physically and functionally differs from the remainder of the DNA. Satellite DNA may contain an appreciable number of odd bases. The nature and quantity of such odd bases may relate to the cellular function of satellite DNA (Cohen, 1967).

The presence of such a multiplicity of rRNA cistrons contained within a unique fraction of the genome of higher plants is intriguing. Of particular interest is that certain mature plants contain a satellite component of DNA while others are lacking in such a component (Matsuda and Siegel, 1967). No quantitative or definitive qualitative relationship has been shown between satellite DNA and physiological function applicable to all plant species, or to a particular plant throughout its life cycle. Also, the intracellular location, as well as the exact nature of the genesis of satellite DNA, remains uncertain.

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