COMPLEXITY OF HUMAN SATELLITE A DNA

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SUMMARY: Satellite A DNA of human origin having a molecular weight of eight million daltons has been isolated. This DNA species has a 51% G+C content and is composed of two kinetically distinct reassociating components. On the average the fast component is repeated 1600 times more frequently than the slow component. Nucleolar DNA appears to be significantly enriched in this satellite.

INTRODUCTION: The DNA extracted from most organisms forms a single band in buoyant CsCl density gradients. The DNA of several mammalian species yield asymmetric bands as a result of the presence of satellite DNAs with different densities which are only partially resolved from the main band (1-5). Differences in DNA densities could be due to G+C content, the presence of unusual bases, or strong binding with other biological macromolecules, e.g. polysaccharides, proteins, or RNA. Most satellite bands from human cells are not well resolved by centrifugation in neutral CsCl density gradients; however, hidden or cryptic satellites may be separated from main band DNA by the formation of heavy metal complexes. Mercury or silver ions are usually employed in conjunction with equilibrium Cs_2SO_4 gradient centrifugation. Corneo, et al., (6-8) have isolated three light human DNA satellites and we have previously isolated a heavy satellite (9) using this method. A nucleolar heavy satellite DNA of buoyant density ρ=1.712 g/ml was found in HeLa cells by Schildkraut and Maio (10). The present work reports the isolation and characterization of a heavy satellite DNA from the nucleolus of human cells designated as satellite A. Satellite A and the Schildkraut-Maio satellite have similar buoyant densities in neutral and alkaline CsCl solutions, so it is possible these satellites DNAs may be identical.

MATERIALS AND METHODS:

DNA Purification: DNA was isolated from human placenta by the technique of Kirby and Cook (11) or Marmur (12). Initially we used sevag extraction (24:1, chloroform: isoamyl alcohol) to minimize single strand breaks in the DNA. Equivalent results were however obtained when freshly distilled phenol was used.

Spectrophotomeric analyses: The UV absorbance spectra, thermal denaturation and renaturation profiles of satellite DNA were determined using a Zeiss PMQ II recording spectrophotometer equipped with a thermostatically controlled cuvette chamber and automatic sampler changer.

Thermal denaturation was carried out in 0.12 M phosphate buffer, pH 6.8. Phosphate buffer was composed of equimolar amounts of NaH_2PO_4 and Na_2HPO_4. The temperature was increased at a constant rate of 0.5° per minute using a Lauda linear temperature programmer. After melting was complete, the temperature was adjusted to 60° to allow reassociation of the DNA. A computer program originally written by Dr. Roy Britten was used to resolve components of the reassociation curve (Dr. James Bonner, personal communication, 1971). The components are best fitted to second order kinetic curves.

Isolation of DNA satellite: A human satellite DNA was isolated from purified native placental DNA by a modification of the technique described by Jensen and Davidson (13). The DNA was sequentially dialyzed against Milipore (0.45 μ pore size) filtered solutions of 0.1 M EDTA, pH 8.0; 0.1 M $NaClO_4$, pH 6.0; 0.1 M Na_2SO_4, pH 6.0; and borate buffer (5 mM $Na_2B_4O_7$, 0.1 M Na_2SO_4, pH 9.1). Freshly prepared $AgNO_3$ (1 mg/ml) was added dropwise to the DNA solution to the desired Ag/PO_4 ratio with constant stirring.

Bentonite treated Cs_2SO_4 was used for the gradients. The initial density was adjusted to 1.55 g/ml by mixing saturated Cs_2SO_4 with the DNA solution and corrected to the desired refractive index according to the equation of Vinograd and Hearst (14).

The final DNA concentration was 200-250 μg/ml. The gradient was centrifuged

in polycarbonate tubes in a Beckman 60 Ti Rotor at 35,000 rpm for 48 hours at 15°. The resulting equilibrium gradient was removed from the tube top using a Buchler Auto Densi-flow and fractionated with absorbance at 254 nm recorded using an ISCO UA-2 Ultraviolet Analyzer and continuous recording device.

The fractions containing satellite DNA were pooled and dialyzed against 5 M NaCl to remove Ag^+ and then sequentially dialyzed as above and centrifuged again in Ag^+-Cs_2SO_4 gradients. The procedure was repeated a third time after which the satellite DNA banded in a homogenous peak in neutral CsCl in the analytical ultracentrifuge.

Ultracentrifugation analysis: Neutral and alkaline CsCl and Ag^+-Cs_2SO_4 gradients were run in a Beckman Model E Analytical Ultracentrifuge. The molecular weight of whole placental DNA and satellite DNA were determined by boundary sedimentation analysis in the analytical ultracentrifuge as described by Studier (15).

RESULTS

(a) Ag/PO_4 ratio, r_f: Purified human bulk DNA was complexed with different amounts of Ag^+ and centrifuged at 35,000 rpm to equilibrium in Cs_2SO_4 density gradients. In each case (figure 1) a satellite shoulder appeared on the light side of the main band. The separation of the satellite band from the main band was found to be improved as the silver to DNA phosphate ratio was adjusted from 0.20 to 0.40. At pH 9.1 the buoyant density of main band DNA-Ag^+ complex increases approximately linearly with the amount of Ag^+ added, while the density of satellite DNA-Ag^+ complex increases with a reduced slope. Thus, at higher r_f (0.4-0.5) satellite band is readily resolved from the main band DNA. The preparative Ag^+-Cs_2SO_4 gradients were heavily loaded with DNA leading to the possibility that initially some of the satellite band was main band DNA. The repeated centrifugation steps and high buoyant density of the satellite DNA would have eliminated any such contamination. Satellite A comprises 0.5-1.0% of the total DNA.

(b) Buoyant density of satellite A DNA: Purified satellite A DNA has a narrow

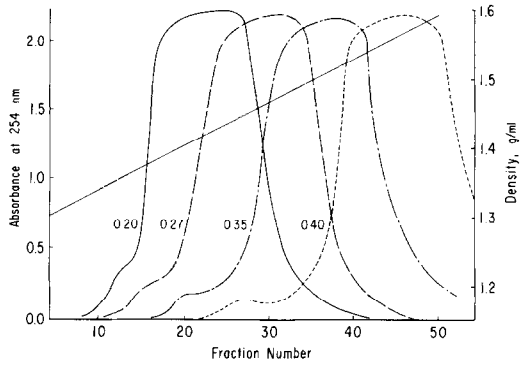

Figure 1: Human DNA in Ag^+-Cs_2SO_4 density gradient. Satellite A DNA is seen as a shoulder on the light side of the gradient. The resolution of the satellite is improved as the Ag^+/DNA phosphate ratio is increased from 0.20 to 0.40. Before centrifugation each gradient was loaded with 200 μg/ml total placental DNA. Centrifugation was in a Beckman 60 Ti Rotor at 35,000 rpm for 48 hours at 15°. The fractions containing satellite DNA were pooled and dialyzed sequentially and centrifuged again in Ag^+-Cs_2SO_4 gradients with Ag/PO_4 ratio of 0.40. The procedure was repeated a third time after which the satellite DNA banded in a homogeneous peak in neutral CsCl in the analytical ultracentrifuge (See figure 2B).

distribution of sedimentation coefficients with a mean of $S_{20,w}$ of 21.6s. This sedimentation coefficient corresponds to a molecular weight of 8 million daltons (15). In neutral CsCl density gradient centrifugation the purified satellite A prepared either from bulk DNA or nucleolar DNA shows a single sharp band at 1.710 g/ml, corresponding to 51% G+C content (figure 2B). The sharpness of the satellite band is indicative of a high molecular weight DNA, homogeneous in base composition.

The density shifts to 1.723 g/ml when the satellite A DNA is heat denatured (figure 2C), and bands at 1.715 g/ml after it is partially reassociated to Cot = 7 (figure 2D). The formation of a single band at both denatured and partially reassociated states suggests that the reassociated sequences are interspersed with unrenatured sequences along each molecule. Alternatively, these densities could have resulted from uniformly reassociated duplex with significant content of mismatched bases.

In alkaline CsCl density gradients satellite A forms a broad symmetric band with a mean density of 1.775 g/ml. This indicates little difference in G+T content between the strands.

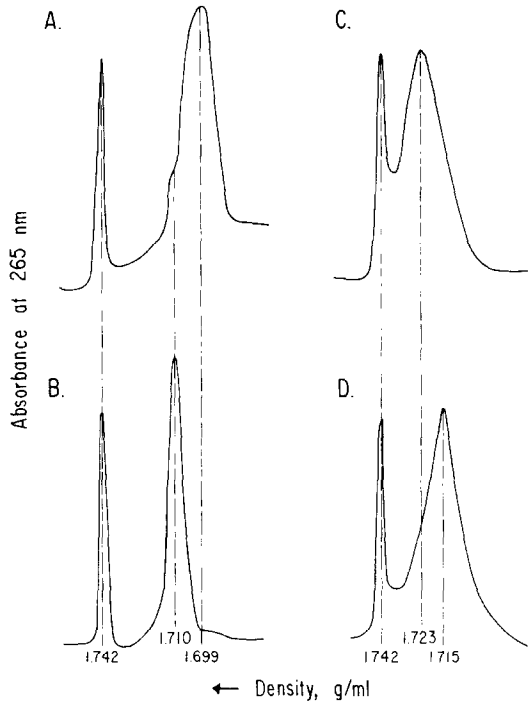

Figure 2: Buoyant density determinations of various satellite DNA preparations. Analytical ultracentrifugation in neutral CsCl of total nucleolar DNA is shown in Panel A. Main band has a density of 1.699 g/ml. Satellite A DNA is seen as a shoulder. Panel B shows purified satellite A DNA by repeated Ag^{+}-Cs_2SO_4 density gradients from either total bulk DNA or nucleolar DNA banding at 1.710 g/ml. Panel C shows satellite A DNA after heat denaturation. Panel D shows satellite A DNA heat denatured and partially reassociated to a Cot of 7. In each case the marker is phage 2C DNA with a buoyant density of 1.742 g/ml. 0.5 μg of marker DNA was added to 1-2μg of the sample DNA. Centrifugation was at 42,040 rpm at 25° for 24 hours. Heat denaturation of satellite A DNA was in 0.12 M phosphate buffer pH 6.8. Partial reassociation was in the same buffer at 60°.

(c) Cellular location of satellite A DNA: To determine if the satellite DNA observed in figure 1 is found in cell nucleoli, the DNA extracted from purified nucleoli of human placenta was centrifuged in $Ag^{+}Cs_2SO_4$ density gradients in parallel with the bulk DNA previously used. A distinct peak corresponding to satellite A plus a slight shoulder corresponding to satellite B (ρ=1.726 g/ml in neutral CsCl density gradient, Chuang, unpublished results) appeared on the light side of the main band of nucleolar DNA (figure 3). Also, the total nucleolar DNA in the CsCl gradient shows a shoulder (figure 2A) corresponding

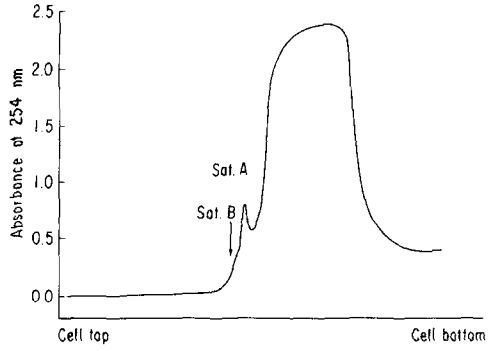

Figure 3: Human nucleolar DNA, banding in $Ag^{+}Cs_2SO_4$ density gradient. DNA isolated from placental nucleoli was centrifuged in Ag^{+}-Cs_2SO_4. Centrifugation was described in the legend of Figure 1 with the optimun Ag/PO_4 ratio of 0.40. Satellite A has a buoyant density of 1.710 g/ml while satellite B has a buoyant density of 1.726 g/ml in neutral CsCl.

to satellite A, while in the case of total bulk DNA this shoulder is not seen. This indicates that nucleoli are enriched in satellite A DNA as was also observed by Schildkraut and Maio (10) with the HeLa cell satellite DNA.

(d) Thermal denaturation profiles: When satellite A DNA is heated in 0.12 M PO_4, pH 6.8 ($[Na^{+}]$ =0.18), it gives a sharp cooperative melting profile with 30% hyperchromicity (figure 4). The Tm is 88.2° which is 5° higher than that of the corresponding native total bulk DNA (figure 4). If the total bulk DNA has 40% G+C content, then the satellite A has 52% G+C content calculated by the relation between Tm and G+C (16), and has 51% G+C calculated by the relation between ρ CsCl and G+C (17). Also, spectral analysis of the denatured DNA (18) showed that the satellite A is 10% higher in G+C than the bulk DNA.

The melting profile of the sheared reassociated (Cot=30) satellite A DNA is rather broad (figure 4). It appears to be non-cooperative at temperatures below 80° and cooperative above 80° with a total hyperchromicity of 21% and Tm of 82.6°. The loss of hyperchromicity and the presence of non-cooperative behavior are probably due to single stranded fractions in the reassociated satellite at Cot 30 (figure 5). If the Tm is dropped by 1.5° by shearing (figure 4) then the sheared reassociated satellite A DNA melts only 4° lower than the

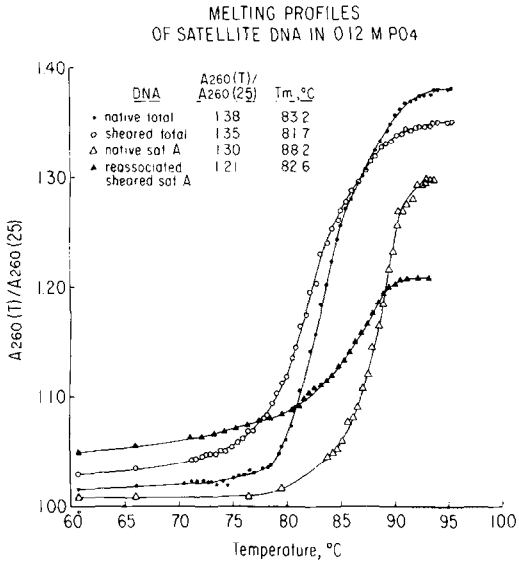

Figure 4: Thermal denaturation profiles of satellite A DNA and total DNA in 0.12 M sodium phosphate, pH 6.8, $[Na^+]$=0.18 M. Sheared satellite was reassociated at Cot = 30 prior to the thermal denaturation analysis.

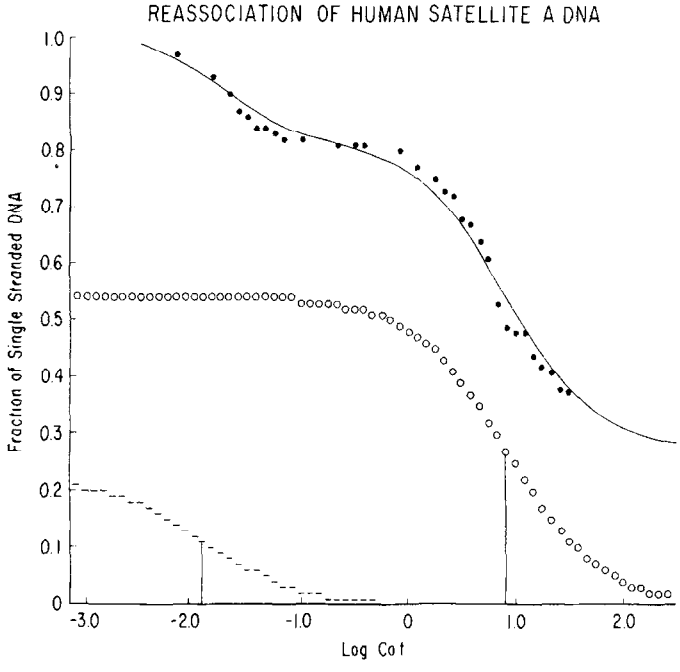

Figure 5: Reassociation of human satellite A DNA. Purified satellite A DNA was sonicated to an average chain length of 250 base pairs, denatured in 0.12 M phosphate buffer pH 6.8, and reassociated at 60°. The reassociation was followed spectrophotomerically at 260 nm with a Zeiss PMQ II spectrophotometer. Experimental points were calculated from the absorbance at 260 nm and best fitted to a second order kinetic curve. The curve was resolved into two components, fast (--) and slow (-00-) by a computer program.

corresponding sheared satellite, i.e. on the average the former contains 3% mismatched base pairs (19).

(e) Reassociation of Satellite A DNA: Satellite A DNA was sonicated to an average length of 250 nucleotides, heat denatured and then allowed to renature. The reassociation was followed spectrophotometrically. The experimental points were then fitted to a second order kinetic curve shown in figure 5. The curve appears to be biphasic and is resolved into two components. The fast component, comprising 22% of the total, reassociates with an apparent $Cot_{\frac{1}{2}}=1.3x10^{-2}$ mole nucleotide sec/liter. The slow portion comprises 54% of the total with an apparent $Cot_{\frac{1}{2}} = 8.3$. However, if each component is isolated in pure form, then the $Cot_{\frac{1}{2}}$ for the fast component is $2.8x10^{-3}$, the $Cot_{\frac{1}{2}}$ for the slow component is 4.5. The fast component is therefore 1600 times more frequently repeated than the slow component.

DISCUSSION: The major findings of this study are: (1) human satellite designated as satellite A was isolated in high molecular weight form, $8x10^6$ daltons; (2) nucleoli are enriched in satellite A DNA; (3) satellite A DNA strands contain two kinetically distinct reassociating sequences; (4) the reassociated DNA has 3% divergence in base sequence as indicated by the reduced thermal stability of reassociated structures.

Interspersed repetitive and non-repetitive sequences were found in the DNA of Drosophila (20,21) and Xenopus (22). It was proposed that the interspersion of repetitive units with non-repetitive protein specifying sequences plays a role in the regulation of gene activity. Satellite A DNA strands contain two kinetically distinct reassociating sequences, but no evidence regarding sequence arrangement was obtained. Studies on the in vivo transcription of the rapidly reassociating component of satellite A will be presented in a separate communication.

The biological functions of satellite DNA remain a mystery. The possibility that satellite DNAs serve a structural function has been suggested by the centromeric location of satellite DNA. The human satellites, however, are not present in all heterochromatins. Human chromosomes contain highly repetitive

sequences similar to satellite DNA on their centromeric and telomeric regions (23). All human chromosomes do not however contain equivalent families of satellite DNA sequences. Chromosome localization studies of satellite A DNA by DNA-RNA hybridization showed no localization of the satellite DNA (presumably the rapidly annealing component), but a wide distribution of grains throughout the chromosomes (Chuang and Arrighi, unpublished results).

We previously reported the isolation of human satellite C DNA (9). This satellite has a buoyant density in neutral CsCl solution (ρ=1.703 g/ml) near the main DNA band (ρ=1.699 g/ml) and is localized in the centromeric heterochromatin of chromosomes C9. In these respects satellite C is quite different from satellites A and B. Satellites C and II have some similarity in chromosome locations; satellite II is localized at A1, E16 and less conspicuously at C9, but have much different base compositions (44% G+C and 34% G+C (7), respectively) computed from both thermal denaturation and buoyant density measurements. A summary of the properties of all human satellite DNAs has recently been presented (25).

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