HETEROGENEITY OF MOUSE SATELLITE DNA ON SILVER–CESIUM SULPHATE DENSITY GRADIENT

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1. Introduction

Mouse satellite DNA constitutes about 8% of nuclear DNA and it is perhaps the most thoroughly studied fraction of repetitive DNA. Mouse satellite DNA is usually purified in its native state by cesium chloride or silver–cesium sulphate density gradient centrifugation using DNA with mol. wt $3–7 \times 10^6$ [1,2].

These preparations are considered to be composed of a highly homogeneous population of DNA molecules. We report here an analysis of mouse satellite DNA in the mol. wt range $4–6 \times 10^6$ using centrifugations in high-resolution silver–cesium sulphate density gradients. This approach has enabled us to resolve mouse satellite DNA into three components.

2. Materials and methods

Mouse DNA was prepared from liver, spleen and cultured mouse skin fibroblasts by three different procedures according to Marmur [3], Walker [4] and Kirby [5]. Occasionally DNA was further purified by preparative centrifugation in CsCl density gradient or by chromatography on columns of methylated albumin–Kieselguhr [6,7]. The sedimentation velocities of the DNA preparations were determined and the molecular weights were calculated according to Eigner and Doty [11].

Analytical silver Cs_2SO_4 density gradients were carried out centrifuging a mouse liver DNA solution (A_{260} 1.200) in 5 mM tetraborate buffer, pH 9.2, containing $AgNO_3$ (Ag/P 0.24) and Cs_2SO_4 ($\varphi = 1.45$ g/cm^3) at 44 770 rev/min at 25°C in an analytical ultracentrifuge Beckman Mod. E. Ultraviolet absorption photographs were taken after 24 h centrifugation, using Kodak professional films. Analytical neutral and alkaline CsCl density gradients were carried out as previously reported [7]. Preparative Ag^+/Cs_2SO_4 density gradients were carried out in a Spinco L 265 B preparative ultracentrifuge using a Spinco type 65 fixed angle rotor. Centrifugation times, speed and DNA concentrations are given in the figure legend.

3. Results and discussion

Figure 1 shows an ultraviolet photograph of mouse DNA analytically centrifuged in a silver–cesium sulphate density gradient using Ag/P ratio of 0.24. A new minor component is visible on the heavy side of the satellite DNA. Increasing the Ag/P ratio this new component merges into the satellite DNA. Two consecutive preparative centrifugations of mouse DNA in Ag^+ Cs_2SO_4 density gradients allow purification of this new component (fig.2).

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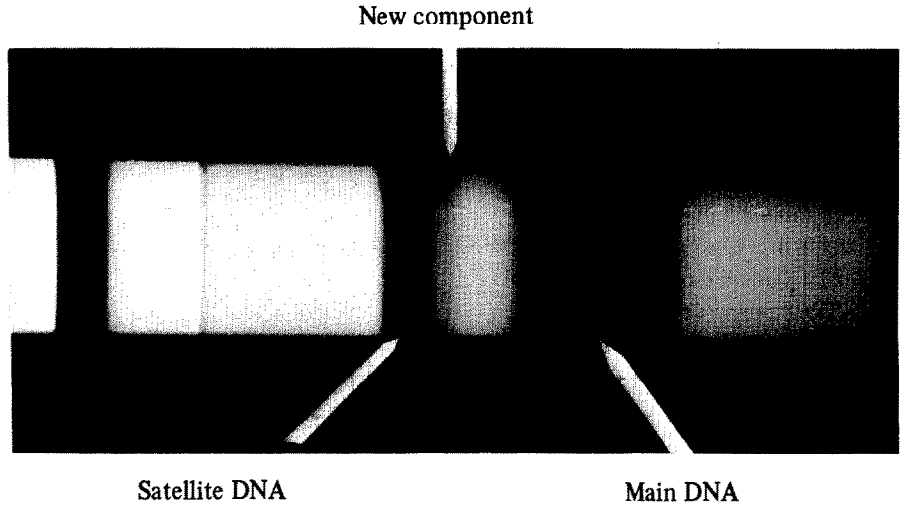

Fig.1. Total mouse DNA centrifuged in silver–cesium sulphate density gradient using an Ag^+/P ratio of 0.24. The new DNA component can be seen between the bands of the main and satellite DNA. For experimental procedures see Materials and methods.

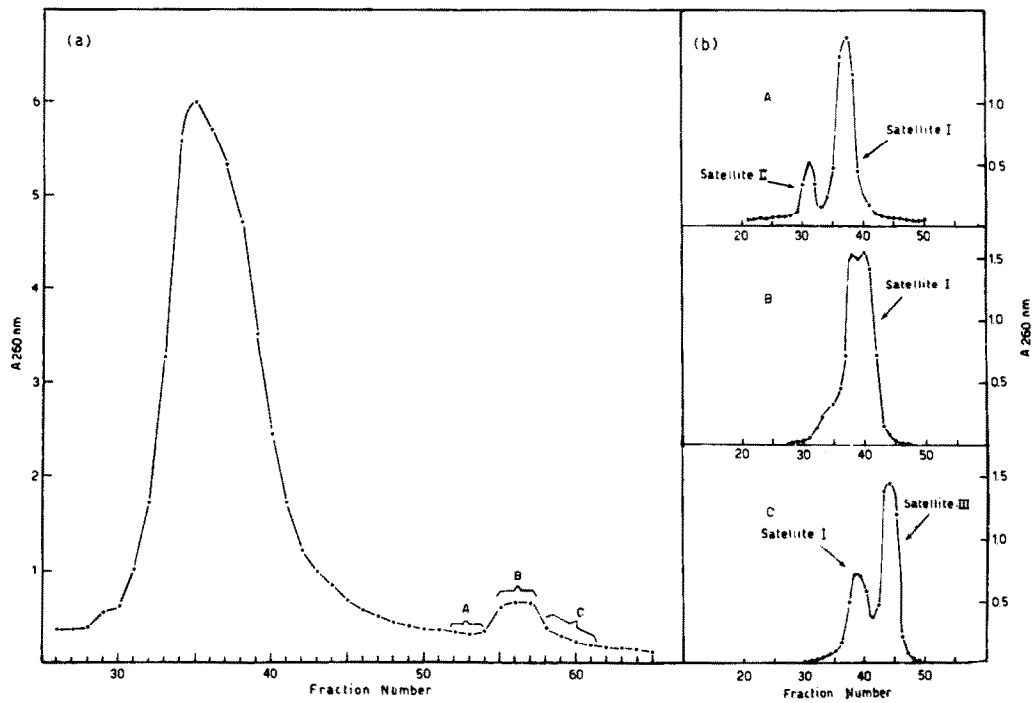

Fig.2. Analysis of mouse DNA using preparative silver Cs_2SO_4 density gradient centrifugations. (a) Mouse liver DNA solution (A_{260} 1.200) in 5 mM tatraborate buffer, pH 9.2, containing $AgNO_3$ (Ag/P 0.24) and Cs_2SO_4 ($\varphi = 1.5$) was centrifuged in a Spinco type 65 fixed-angle rotor at 38 000 rev/min for 70 h and at 28 000 rev/min for an additional 25 h to reduce the slope of the gradient [9,10]. Fractions of 0.20 ml were then collected from the top of the tube and the A_{260} measured in a Gilford 240 spectrophotometer. The material obtained was seperated into fraction A, B and C. Corresponding fractions obtained from 8 tubes simultaneously centrifuged were pooled together. (b) Fractions A, B and C were adjusted to a density of 1.5 g/cm^3 with Cs_2SO_4 and rerun under identical centrifugation conditions. Small amounts of these samples were also centrifuged in an analytical ultracentrifuge Beckman Mod. E at 44 770 rev/min for 24 h at 25°C and produced a DNA distribution identical to that observed in preparative centrifugations.

Furthermore by rebanding, under identical centrifugation conditions, the fractions collected from the light side of the satellite DNA we were able to resolve a third component (fig.2). We call the heavy component mouse satellite II, and the light component mouse satellite III.

We have roughly estimated that component II makes up about 10% of the total mouse satellite DNA and the component III 20%. We obtained identical results with mouse DNA prepared from liver, spleen or cultered mouse skin fibroblasts and with DNAs prepared by three different procedures (according to Marmur [3], Walker [4], Kirby [5]). In some cases DNA was further purified by preparative centrifugation in CsCl density gradient or by chromatography on columns of methylated albumin–Kieselguhr [6–7]. In all cases we were able to resolve mouse satellite DNA into three components.

All these three purified mouse satellite DNA components, after removal of Ag^+ by extensive dialysis in 2 M NaCl show on analytical centrifugation in neutral CsCl density gradient a single peak having a density of 1.691 g/cm^3 (*E. coli* DNA = 1.710). In preliminary experiments with the purified satellite DNA components we were unable to show differences between satellites I and III in melting point or density of the two strands on alkaline CsCl gradient centrifugation. On the other hand no strand separation was observed when satellite II was centrifuged in alkaline CsCl density gradient. These data suggest that mouse satellite DNA, purified by the usual procedures, at mol. wt $4–6 \times 10^6$ daltons, is a mixture of at least three different components having similar density in gradients of cesium chloride.

Clearly, since two of these three components can be differentiated only by virtue of small density differences on silver–cesium sulphate gradients, further parameters are needed in order to confirm this macro-heterogeneity of mouse satellite DNA. However these observations could explain some of the peculiar characteristics of mouse satellite DNA [7,8].

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