SATELLITE DNA IN MOUSE AUTOSOMAL HETEROCHROMATIN

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Within the last several years numerous investigations have been concerned with the existence of a minor DNA component, termed satellite DNA, in the genome of a number of higher organisms (Kit, 1961; Chun and Littlefield, 1963; Corneo et al., 1968; Britten and Kohne, 1968). In the present communication, we wish to report that mouse autosomal heterochromatin, isolated by sonication of purified nuclei, is composed primarily of satellite DNA.

EXPERIMENTAL

Liver nuclei from young adult male mice (Swiss Webster) were isolated in heavy sucrose by a method similar to that of Løvtrup-Rein and McEwen (1966). The isolation of autosomal heterochromatin and euchromatin was achieved by a modification of the method described by Frenster et al. (1963): The nuclei are suspended in 0.25 M sucrose and allowed to swell by standing for 10 minutes at 4° C. They are then disrupted by sonication for 10 seconds in an S-125 Branson sonifier at 6 amps and 20,000 cycles per second. The resulting suspension of chromatin is composed of 3 fractions; a heterochromatin fraction which is separated by sedimentation at 3500g for 20 minutes, an intermediate fraction of euchromatin contaminated with small amounts of heterochromatin, which is separated by sedimentation at 78,000g for 1 hour and a euchromatin fraction which is precipitated by the addition of two volumes of alcohol. Preparations of nuclei, heterochromatin and euchromatin were monitored for purity by phase contrast microscopy and by light microscopy using Wright's

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stain.

DNA from heterochromatin and euchromatin was extracted by deproteinization with chloroform:isoamyl alcohol and the RNA removed with heat treated pancreatic RNase as described by Marmur (1961). DNA base composition was determined by hydrolyzing the DNA with 90% formic acid (Wyatt and Cohen, 1953), and separation of the bases by high voltage electrophoresis as described by Efron (1960). The spots were marked under UV light, eluted for 24 hours in 0.1 N HCl and the optical density of the eluates measured at the wavelength of maximal absorption of the respective bases.

Cesium chloride gradient centrifugation was performed at neutral pH according to Flamm et al. (1966). The buoyant density was calculated from the equation relating density to refractive index (Vinograd and Hearst, 1962).

RESULTS

Cesium chloride sedimentation patterns of total DNA and DNA from the heterochromatin and euchromatin fractions of mouse liver are shown in Fig. 1.

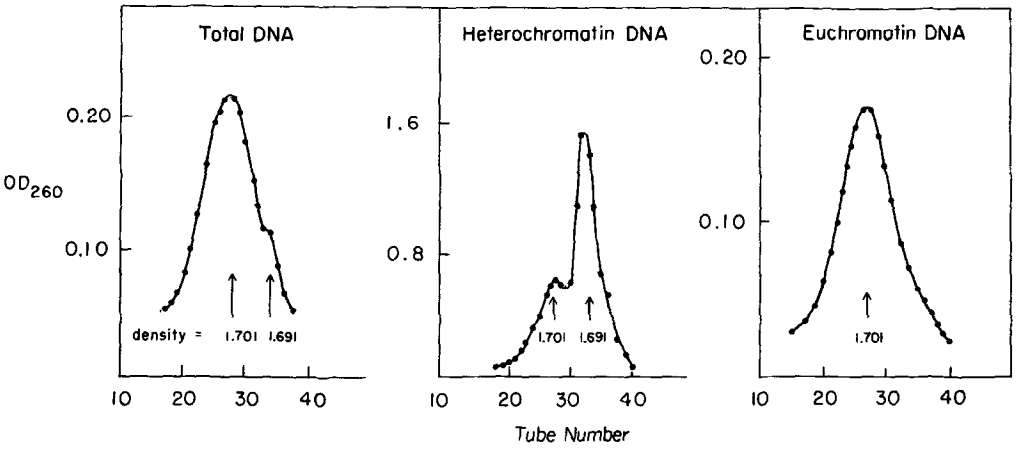

Fig. 1. CsCl sedimentation patterns of total DNA and of DNA from the heterochromatin and euchromatin fractions of mouse chromatin. The amounts of nucleic acid applied were 0.7, 4.0, and 0.6 OD_{260} units, respectively. Following centrifugation as described under methods, the tubes were pierced with a 21 gauge needle and 10 drops aliquots (0.1 ml) were collected, diluted with 0.2 ml of 0.01 M Tris buffer, pH 8.2, and the optical density measured at 260 mμ in a Beckman DU spectrophotometer.

Total DNA reveals a major peak of euchromatin at a density of 1.701 and a minor peak (about 10%) of satellite DNA at a density of 1.691. DNA from the heterochromatin fraction shows the same two peaks, but is composed mainly of satellite DNA (about 70%). Since heterochromatin DNA comprised 10% of total DNA in these experiments, this represents 70% of the total amount of satellite DNA in the nucleus. DNA from the euchromatin fraction shows only one peak at a density of 1.701.

To verify the identity of the DNA in the major peak of the heterochromatin fraction, its base composition was determined as shown in Table I. DNA from the major peak yielded a base ratio of 2.00 as compared to a value of 1.49 for total DNA, in agreement with the values reported in the literature for mouse satellite DNA and total DNA respectively (Corneo et al., 1968).

Table I. Base composition of total DNA and of satellite DNA from mouse liver.

Fraction	%A	%T	%G	%C	$\frac{A+T}{G+C}$
Total DNA	29.2	30.6	19.8	20.4	1.49
Heterochromatin DNA* (major peak)	34.2	32.8	16.7	16.3	2.00

* The fractions in the major peak of heterochromatin DNA (as shown in Fig. 1) were pooled, dialyzed extensively against SSC buffer (0.15 M NaCl, 0.015 M sodium citrate, pH 7.1), and the DNA recovered by precipitation with two volumes of ethanol.

DISCUSSION

The observation that autosomal heterochromatin from mouse liver nuclei is composed primarily of satellite DNA is quite interesting in view of the fact that, to date, no definite role has been established for either of these entities. Since satellite DNA has been shown to be present in various mouse tissues in approximately the same proportions (Kit, 1961; Chun and Little-

field, 1963), it is quite probable that this observation can be extended to other tissues. Preliminary findings in our laboratory indicate that heterochromatin from mouse brain nuclei is also rich in satellite DNA. The further extension of this observation to other species may aid in elucidating the role of autosomal heterochromatin.

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