easily visualized as being initially heterogeneous. For instance, troponin could perhaps be preferentially phosphorylated by one subunit and phosphorylase and glycogen synthase by another. Alternatively, all catalytic sites could initially be physically and catalytically homogeneous but become catalytically heterogeneous after reacting with the oATP. This could occur, for instance, if a modified site was still capable of phosphorylating certain substrates (the troponins), but not others (phosphorylase and glycogen synthase). Yet another explanation for our data could be the binding of the oATP affinity label to a regulatory site, resulting in conformational changes at a single type of catalytic site which are then expressed differently with different protein substrates. Experiments currently under way in our laboratory with other adenine nucleotide affinity labels capable of forming stable, isolable covalent linkages should help to distinguish the correct mechanism from among these various possibilities.

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Interspersion of Mouse Satellite Deoxyribonucleic Acid Sequences†

Peter J. Stambrook

ABSTRACT: DNA sequences with homology to the major (A + T)-rich mouse satellite component were localized in CsCl gradients by hybridization with a labeled satellite cRNA probe. Although, as expected, most of the hybridization was to DNA in the satellite-rich shoulder, substantial radioactive cRNA hybridized with DNA from denser regions of the gradient. Further examination revealed that hybridization to main-band DNA was not due to physical trapping of satellite DNA in the gradient, and melting experiments argue that the associated radioactivity was due to true RNA/DNA hybridization. Nearest-neighbor analysis of hybridized [α-^{32}P]CTP-labeled l-strand cRNA indicates that hybridization to main-band DNA is by the satellite cRNA and not a contaminant. Together, these data argue that mouse satellite-like sequences are interspersed within the main-band fraction of DNA. For the support of this contention, total mouse DNA, purified main-band DNA, and purified satellite DNA were digested with *Eco*RI, sedimented in a sucrose gradient, and hybridized with labeled satellite cRNA. Mouse satellite DNA is not cleaved with *Eco*RI, so that purified *Eco*RI-digested satellite DNA sediments as a high molecular weight component. When total mouse DNA is digested with *Eco*RI, the majority of satellite-like sequences remain as high molecular weight DNA; however, significant amounts of satellite-like sequences sediment with the bulk of the lower molecular weight digested DNA, lending further credence to the argument that satellite-like sequences are interspersed with main-band DNA.

Nuclear satellite DNAs are characteristic of most, if not all, higher eukaryotes [for review, see Skinner (1977) and John & Miklos (1979)]. These DNAs are comprised of highly repeated nucleotide sequences ordered in a tandem array. They vary in complexity, ranging from the very simple sequences comprising the crab poly[d(A-T)] satellite (Sueoka & Cheng, 1962) and the heptanucleotide repeats found in *Drosophila virilis* (Gall & Atherton, 1974) to components with a periodicity as great as 1400 base pairs (bp) such as calf

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satellite I (Botchan, 1974; Maio et al., 1977). In mouse, the major satellite is (A + T)-rich, comprises about 6% of the total genome, and is arranged as a tandem repeating unit with a periodicity of about 240 bp (Southern, 1975; Hörz & Zachau, 1977). In situ hybridization experiments have localized satellite DNA sequences at the centromere of every chromosome except possibly the Y chromosome (Jones, 1970; Pardue & Gall, 1970). In situ hybridization, which clearly shows that centromeric regions in mouse are rich in satellite DNA, does not, however, exclude the possibility that the same satellite sequences or very closely related sequences may occur at sites other than at centromeres and in an organization other than in a repeating tandem array. There is a precedent which argues that not all mouse satellite DNA sequences are strictly located at the centromere. Bostock & Clark (1980) have described a methotrexate-resistant mouse cell line in which the genes coding for dihydrofolate reductase are extensively amplified. Satellite DNA sequences, apparently associated with this noncentromeric gene, had undergone concomitant amplification. In other organisms such as the red crab *Geryon quinquedens*, highly repetitive DNA, which may exist in part as a cryptic satellite, is also interspersed with single-copy DNA (Christie & Skinner, 1979). The experiments described in this report argue that although mouse satellite DNA sequences are known to exist primarily as tandemly repeated units, they may not be confined solely to this clustered organization, but may also be interspersed with nonsatellite DNAs.

Materials and Methods

Preparation of Satellite and Main-Band DNAs. The source of the unlabeled DNAs was from CBA and C57 mouse livers. Radioactive mouse DNA was obtained by continuously labeling 3T3 cells with [^{3}H]thymidine for 72 h. In each case, the DNAs were isolated by a protocol modified from one previously described (Stambrook, 1974). Livers were minced and incubated for 1 h at 37 °C in buffer containing 0.15 M NaCl, 0.1 M EDTA, 1% sodium lauryl sulfate, and 50 μg/mL proteinase K (E. M. Merck). Radiolabeled tissue culture cells were lysed in the same buffer and subjected to the same 1-h proteinase K digestion. Samples were gently shaken overnight at 4 °C with an equal volume of phenol that had been saturated with 0.1 M sodium borate (pH 9.2). After a second phenol extraction, the DNA was precipitated, redissolved in 0.1 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate), and subjected to RNase digestion (50 μg of pancreatic RNase/mL and 50 units of T_1 RNase/mL, 1 h at 37 °C) followed by a second proteinase K digestion (50 μg/mL for 1 h at 37 °C) and extensive dialysis against 0.01 M sodium borate. After an additional phenol extraction, the DNA was precipitated and dissolved in 0.01 M sodium borate, pH 9.2.

Separation of satellite DNA from the main band was performed essentially as described by Corneo et al. (1968). The final density of the DNA samples was brought to 1.498 g/cm^3 with solid Cs_2SO_4, after addition of Ag_2SO_4 to give a 0.3 molar ratio of Ag^+ to DNA phosphate. The separation of satellite DNA from main-band DNA was effected by centrifuging the samples for 48 h at 20 °C and 33 000 rpm in either a Beckman 50 Ti or a 60 Ti rotor. The gradients were fractionated and passed through a Gilford continuous flow cell monitored at 260 nm. The satellite DNA, displaced to the light side of the gradient (Figure 1a), was collected, dialyzed against 0.1 × SSC to remove the silver ion, and recentrifuged to equilibrium in neutral CsCl with a starting density of 1.695 g/cm^3. For the elimination of contaminating main-band DNA from the satellite preparation, DNA from the heavy side of the satellite absorbance profile was removed and the sample subjected to yet one more round of centrifugation. Main-band DNA was purified in the same way, but the light side of the absorbance profile was discarded to remove remnants of satellite DNA. The purity of the satellite preparation was assessed in the analytical ultracentrifuge in neutral CsCl, which yielded a homogeneous peak (Figure 1b), and by strand separation in alkaline CsCl, which yielded two approximately equal peaks (Figure 1c).

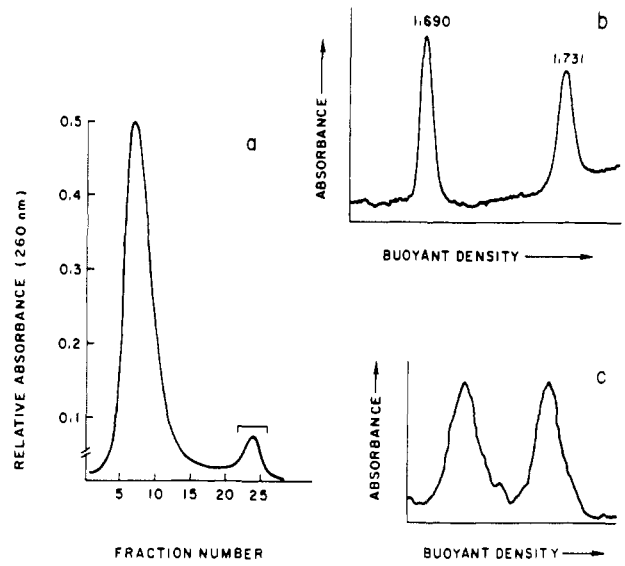

FIGURE 1: Preparation and analysis of mouse satellite DNA. (a) Purified mouse DNA centrifuged to equilibrium in an Ag^+/Cs_2SO_4 gradient. Satellite DNA is displaced to the light side. (b) Analytical ultracentrifugation of purified satellite DNA in neutral CsCl. The density of the satellite is 1.690 gm/cm^3; the density of the *M. luteus* marker DNA is 1.731 gm/cm^3. (c) Analytical ultracentrifugation of 3 μg of purified mouse satellite DNA in alkaline CsCl.

Preparation of the Satellite l Strand and Its Complementary RNA (cRNA). CsCl was added to 20 μg of purified satellite DNA. The sample was brought to 0.1 N NaOH in a final volume of 5 mL and to a refractive index of 1.4042. After centrifugation for 48 h at 33 000 rpm in a Beckman 50 Ti rotor, the tubes were punctured, the gradients were fractionated into 30-drop fractions, and the absorbance at 260 nm was read. The light strand was identified, collected, and neutralized. In vitro transcription of the l strand was performed as described by Reeder & Brown (1970), using either [^{3}H]CTP or [α-^{32}P]CTP as the radioactive nucleotide.

Hybridization of cRNA. The DNA in each fraction was denatured by the addition of 1 mL of 0.1 N KOH. After 10 min, the samples were placed in ice. Each sample was neutralized with 0.4 mL of 10 × SSC containing 0.3 N HCl and 0.5 M Tris-HCl, pH 8.0, and immediately filtered onto a 2.3-mm nitrocellulose disk. Prompt handling of the samples in the cold was necessary to minimize rapid reassociation of the concentrated satellite sequences in the light region of the gradient. The filters were baked overnight at 65 °C and hybridized with satellite cRNA in 50% formamide and 0.6 M NaCl, 1 mM EDTA, and 0.2 M Tris-HCl, pH 7.5, at 38.5 °C for 16 h. The filters were washed 3 times in 2 × SSC, incubated for 1 h with 50 μg/mL pancreatic RNase, washed 4 more times for 15 min each with 2 × SSC, and were dried and counted.

Melting of RNA from Filters. For assessment of the stability of the hybrid formed between satellite l-strand cRNA and the filter-bound DNA, appropriate filters were placed collectively in 1.5 mL of 0.1 × SSC at 45 °C. After 5 min, the SSC was removed and replaced with a fresh 1.5-mL sample of 0.1 × SSC and the temperature elevated to 50 °C. This

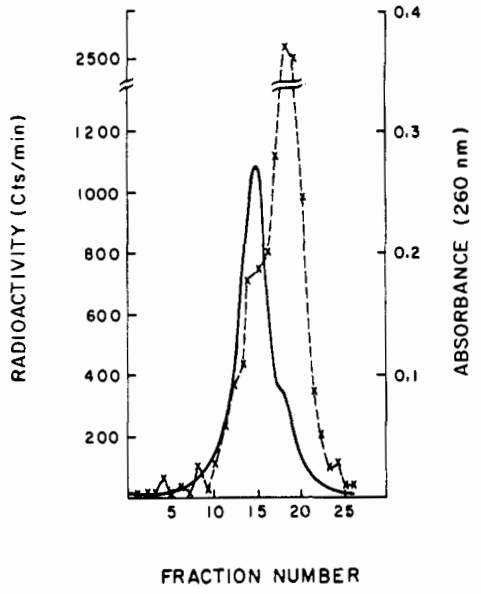

FIGURE 2: Hybridization of satellite l-strand [^{3}H]cRNA to total mouse DNA banded in neutral CsCl. Absorbance at 260 nm (—); radioactivity hybridized (×---×).

precedure was repeated at 5 °C intervals, and the radioactive RNA eluted at each temperature was counted in Aquasol (New England Nuclear).

Distribution of Radioactivity in 2′,3′-Mononucleotides after Alkaline Hydrolysis. Light-strand cRNA labeled with [α-^{32}P]CTP and hybridized to filter-bound DNA was removed from the hybrid by incubating the filters in 0.1 × SSC at 100 °C for 2 min. The RNA was hydrolyzed in 0.3 N KOH, and the 2′,3′-ribonucleotides were separated by electrophoresis on Whatman 52 paper in 5% acetic acid and 0.5% pyridine, pH 3.5, at 3 kV for 1 h. The separated nucleotides were identified by X-ray autoradiography, cut out, and counted.

Restriction Enzyme Digestion and Sucrose-Gradient Analysis. Purified satellite DNA (3 μg), purified main-band DNA (30 μg), and total mouse DNA (30 μg) were digested with 5 units of *Eco*RI restriction endonuclease for 2 h at 37 °C in 50 mM NaCl, 5 mM $MgCl_2$, and 100 mM Tris-HCl (pH 7.5). Aliquots of satellite DNA (1.5 μg), main-band DNA (5 μg), and total mouse DNA (5 μg) were subjected to electrophoresis in a 1% agarose slab gel for 15 h at 15 mA in 5 mM sodium acetate, 1 mM EDTA, and 0.04 M Tris-HCl, pH 7.9. The gel was stained in ethidium bromide (1 μg/mL) and photographed.

The remainder of the DNA samples was layered onto a 10–40% sucrose gradient containing 1 M NaCl, 20 mM Tris-HCl, pH 8.0, and 10 mM EDTA in a Beckman SW 41 rotor. The gradients, which were centrifuged for 18 h at 20000 rpm and 20 °C, were fractionated from the top into 25-drop fractions. The DNA in each fraction was denatured by addition of 2.5 mL of 0.1 N KOH. Immediately prior to adsorption to nitrocellulose filters, the samples were neutralized by addition of 1.0 mL of 10 × SSC containing 0.3 N HCl and 0.5 M Tris-HCl, pH 8.0. The filter-adsorbed DNA was hybridized with ^{3}H-labeled l-strand cRNA, washed, ribonuclease treated, and counted as described above.

Results

Distribution of Satellite DNA Sequences in a CsCl Gradient. Preparative isopycnic centrifugation of high molecular weight mouse DNA in neutral CsCl reveals the major (A + T)-rich satellite as a discrete shoulder on the light side of the absorbance profile (Figure 2). The satellite is comprised of a major repeating unit with a periodicity of about 240 bp

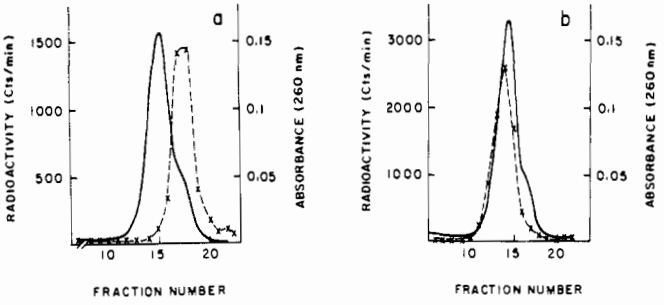

FIGURE 3: Rebanding of mouse satellite and main-band DNAs. (a) ^{3}H-labeled satellite DNA (0.1 Mg) (45000 cpm/μg) was mixed with 25 μg of unlabeled mouse DNA and banded in a neutral CsCl gradient. (b) ^{3}H-labeled main-band DNA (0.2 μg) (42000 cpm/μg) was mixed with 25 μg of unlabeled mouse DNA and banded in a neutral CsCl gradient. Absorbance at 260 nm (—); radioactivity (×---×).

(Southern, 1975). If all the satellite sequences were arranged as tandemly repeating units, hybridization with a labeled satellite probe to DNA in the gradient would be confined to the (A + T)-rich shoulder. Unexpectedly, [^{3}H]cRNA complementary to the light strand of purified satellite DNA (Figure 1) hybridized with DNA in denser regions of the gradient (Figure 2) as well as with DNA in the satellite shoulder. Although this result suggests that satellite-like DNA sequences may be interspersed with DNAs of higher density, several alternative interpretations remain. For example, it is possible that the hybridization to DNAs in dense regions of the gradient is due to physical trapping of the satellite within the gradient. This alternative explanation was examined in three ways. (1) The DNA was sheared through a 25-gauge needle to a size between 5 × 10^6 and 10 × 10^6 daltons before banding in CsCl. Despite this treatment, which predictably resulted in a substantially broadened absorbance profile, the distribution of hybridization within the gradient was not substantially altered (data not shown). (2) Two mixing experiments were performed to independently assess the degree of physical entrapment during the isolation procedure. Radioactively labeled mouse satellite DNA and main-band DNA were prepared from 3T3 cells that had been cultured for 3 days with [^{3}H]thymidine. These labeled DNAs were mixed with mouse liver nuclei as they were being lysed at the start of the extraction procedure. The DNAs were isolated by conventional means and banded in neutral CsCl. As figure 3 illustrates, the radioactive satellite and main-band DNAs band faithfully within the gradient, indicating that physical trapping had not occurred. (3) The labeled satellite DNA probe was hybridized to mouse main-band DNA from which the satellite had been removed. The major satellite component was initially separated from the main band of DNA by centrifugation in an Ag^+/Cs_2SO_4 gradient as shown in Figure 1. The recovered main-band DNA was further purified in neutral CsCl, and (A + T)-rich DNA on the light side of the absorbance profile was discarded to ensure complete removal of the satellite. The remaining DNA was subjected to a final centrifugation and was hybridized with l-strand [^{3}H]cRNA. As Figure 4 illustrates, sequences within the satellite-depleted main-band DNA hybridized effectively with the labeled l-strand cRNA, suggesting that satellite-like sequences were interspersed within the bulk of the main-band DNA.

Melting Profiles and Nucleotide Analyses of the RNA/DNA Hybrids. Although the hybridization reactions were performed under conditions sufficiently stringent to favor stable duplex formation, and although the filters were treated with ribonuclease to digest unhybridized RNA, the possibility remained that the radioactivity associated with main-band DNA was due to nonspecific adsorption rather than faithful hy-

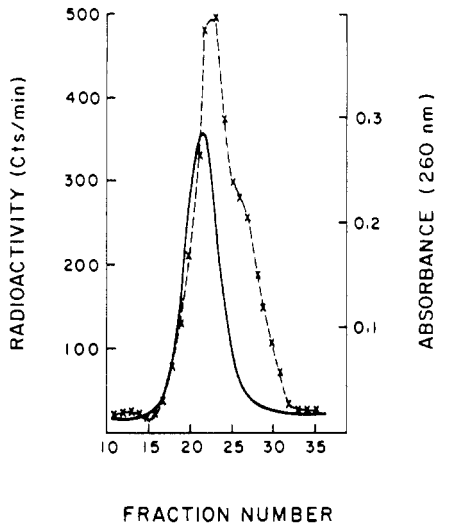

FIGURE 4: Hybridization of satellite l-strand [^{3}H]cRNA to main-band DNA. Main-band DNA, purified by Ag^+/Cs_2SO_4 and subsequent neutral CsCl gradients, was banded in neutral CsCl, and the DNA in each fraction was hybridized with satellite l-strand [^{3}H]cRNA. Absorbance at 260 nm (—); radioactivity hybridized (×---×).

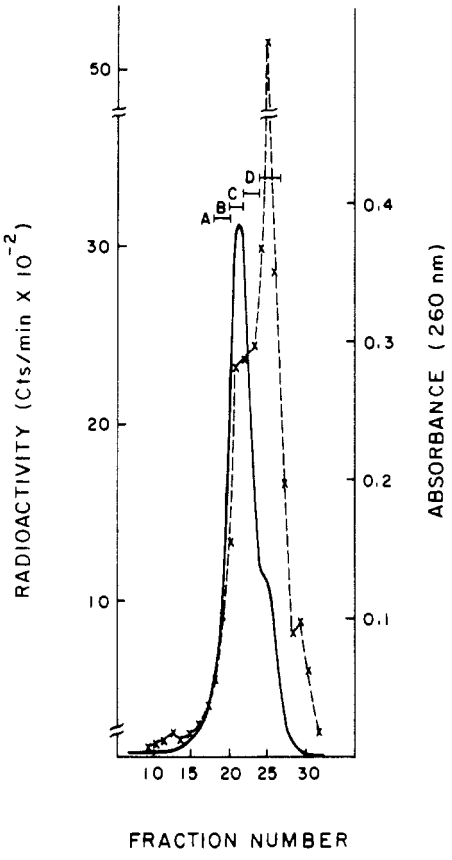

FIGURE 5: Pooled fractions of ^{32}P-labeled satellite cRNA hybridized to mouse DNA. Mouse DNA (50 μg) was banded in neutral CsCl, the DNA in each fraction was denatured and adsorbed to a nitrocellulose filter, and the filters were pooled into four groups (A–D) representing progressively lighter regions of the gradient.

bridization. For assessment of the nature of the bound radioactivity, two identical CsCl gradients containing unsheared mouse DNA were prepared, and the DNA in each fraction was hybridized with an [α-^{32}P]CTP-labeled satellite l-strand cRNA. The asorbance and hybridization profiles of one of the gradients are displayed in Figure 5. The filters from each gradient were combined into four pools designated A through D so that each pool encompassed a range of DNAs with differing (G + C) content (Figure 5). After radioactive

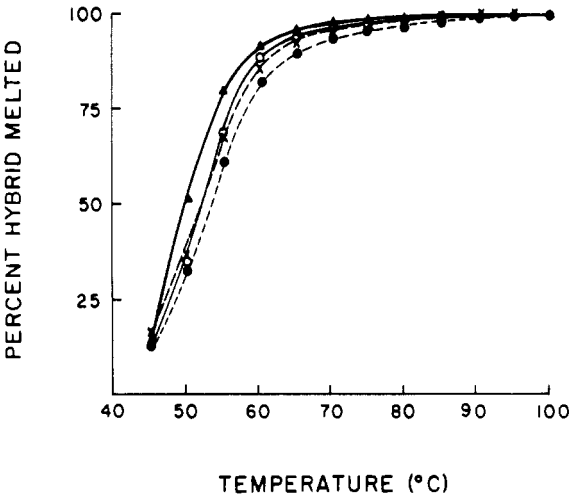

FIGURE 6: Melting profiles of hybridized cRNA. Filters containing hybridized [^{32}P]cRNA from regions A–D of the CsCl gradient depicted in Figure 5 were immersed in 1.5 mL of 0.1 × SSC at 45 °C. The temperature was raised in 5° C increments, and at each temperature, the buffer was removed, counted, and replaced with fresh buffer. Region A (●); region B (▲); region C (×); region D (○).

Table I: Percent of Radioactivity in Each of the 2′,3′-Ribonucleoside Monophosphates after Alkaline Hydrolysis of Hybridized Strand Satellite cRNAs[a]

	2′,3′-mononucleotide			
	U	G	A	C
pooled fractions				
A	30.1	24.5	25.0	20.4
B	34.1	22.5	26.4	17.0
C	33.6	24.6	25.6	16.2
D	32.5	25.8	26.0	15.7
unhybridized cRNA	33.4	20.1	26.9	19.5

[a] Hybridized [α-^{32}P]CTP-labeled cRNAs eluted from pooled filters from fractions A–D depicted in Figure 5 were alkaline hydrolyzed, and the nucleotide products were electrophoretically separated, identified by autoradiography, cut out, and counted. The level of radioactivity in a given spot from hydrolyzed eluted RNA ranged from 75 to 390 cpm.

counting, scintillation fluid was removed from the filters by washing with ether. The filters were dried and submerged in 0.1 × SSC, and the RNA was released from hybrids by raising the temperature in successive 5 °C increments. As Figure 6 illustrates, release of radioactivity from the filters from each pool is temperature dependent, as expected for true RNA/DNA hybrids, and the T_m approximates the T_m of 56 °C predicted for reassociated mouse satellite DNA denatured in 0.1 × SSC (Flamm et al., 1967; Corneo et al., 1968). Furthermore, the melting profile is similar for cRNA hybridized to DNA in all parts of the CsCl gradient, suggesting that in each case the cRNAs are hybridizing with (A + T)-rich sequences like those of the satellite.

In the parallel gradient, RNA was eluted from the filters in each of the pools (A–D, Figure 5) and subjected to alkaline hydrolysis. The nucleotides were separated electrophoretically, and the radioactivity associated with each of the nucleotides was determined. As illustrated in Table I, the nearest-neighbor frequency of nucleotides adjacent to C in RNA eluted from each of the four pools and from unhybridized cRNA is similar, indicating that hybridization to DNA on the dense side of the absorbance profile is not due to a (G + C)-rich contaminant in the probe.

Size Distribution of Satellite Sequences after EcoRI Digestion. A remaining potential source of main-band-associated satellite-like sequences is that of junctional molecules. These

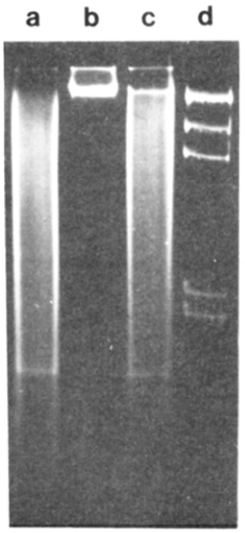

FIGURE 7: *Eco*RI digestion of mouse DNAs. (a) Main-band DNA, (b) purified satellite DNA, and (c) total mouse DNA were digested to completion with *Eco*RI, electrophoresed in a 1% agarose slab gel, and stained with ethidium bromide. Lane d represents a *Hind*III digest of λ-DNA. The small band apparent in lanes a and c is likely a cryptic satellite that is not separated from main-band DNA under the conditions used in these experiments.

would be randomly broken molecules containing some satellite sequences but comprised primarily of adjacent nonsatellite DNAs of sufficiently high (G + C) content to band with the bulk of the main-band sequences. For assessment of the contribution that such molecules may make to the main-band hybridization profile, the DNAs were digested with the restriction enzyme *Eco*RI, which does not cleave mouse satellite DNA with a regular periodicity, leaving it as a high molecular weight component as shown in Figure 7 [see also Southern (1975) and Hörz & Zachau (1977)]. The digestion products were fractionated by sucrose-gradient centrifugation and tested for their ability to hybridize with a labeled satellite cRNA probe. The rationale for this approach is that if satellite DNA exists solely in a clustered configuration, all satellite sequences including those at junctions with nonsatellite DNA should sediment as a high molecular weight species. If satellite-like sequences are also interspersed with nonsatellite DNA, the flanking nonsatellite sequences should contain randomly distributed *Eco*RI sites. Digestion with *Eco*RI should cleave at these sites, rendering the associated satellite a low molecular weight component which should sediment with the bulk of the digested DNA. In agreement with the electrophoretic data (Figure 7c), the sucrose-gradient absorbance profile of total mouse DNA after *Eco*RI digestion shows that the digestion products sediment predominantly with molecular weights between roughly 1 and 15 kilobase pairs (kbp) (Figure 8a). Hybridization with a satellite cRNA (Figure 8b) shows that most of the satellite remains high molecular weight. This is consistent with electrophoretic results depicted in Figure 7b, which indicate that virtually all of the purified satellite remains larger in size than 23 kbp, the size of the largest λ *Hind*III marker fragment. The hybridization profile of total digested DNA, however, reveals that whereas most of the satellite remains substantially larger than 50 kbp (the λ-DNA marker), about 20% of the hybridized radioactive cRNA is associated with the low molecular weight component (Figure 8a). This is in contrast to results obtained for the same type of experiment using purified *Eco*RI-digested satellite DNA where less than 3% of the hybridized radioactivity is low molecular weight (Figure 8b). Hybridization to main-band DNA digested with *Eco*RI was primarily associated with low molecular weight fragments (Figure 8c).

The hybridization profile of satellite cRNA to total mouse DNA digested with *Eco*RI (Figure 8a) is that expected if satellite-like DNAs are interspersed with nonsatellite sequences. The level of hybridization to the low molecular weight sequences is similar to the amount of hybridization to the main-band component of mouse DNA centrifuged in neutral CsCl. In both cases, the percentage of hybridization to the "interspersed fraction" is probably an overestimate since the level of hybridization to the bulk of the satellite sequences is less than expected, possibly because of rapid reassociation before adsorption to nitrocellulose filters.

Discussion

It is clearly established that mouse satellite DNA sequences exist as highly clustered tandem repeats of a 240-bp repeating unit (Southern, 1975) localized at centromeric regions of virtually every chromosome (Jones, 1970; Pardue & Gall, 1970). What remains to be determined is whether mouse satellite DNA occurs exclusively in a clustered arrangement, or whether it is also interspersed with other sequences. An earlier report (Flamm et al., 1969) described experiments suggesting interspersion of satellite sequences within main-band DNA. Subsequently, Cech & Hearst (1976) demonstrated by DNA/DNA reassociation that up to 15% of the mouse main-band DNA contains highly repeated sequences, a small fraction of which have a buoyant density similar to that of the mouse satellite. The nature of the satellite-like sequences and the possibility that they represent a contaminant of the major (A + T)-rich satellite were not further explored. Interspersion of satellite sequences may also be inferred from the observation that mouse cells, resistant to methotrexate because of amplified

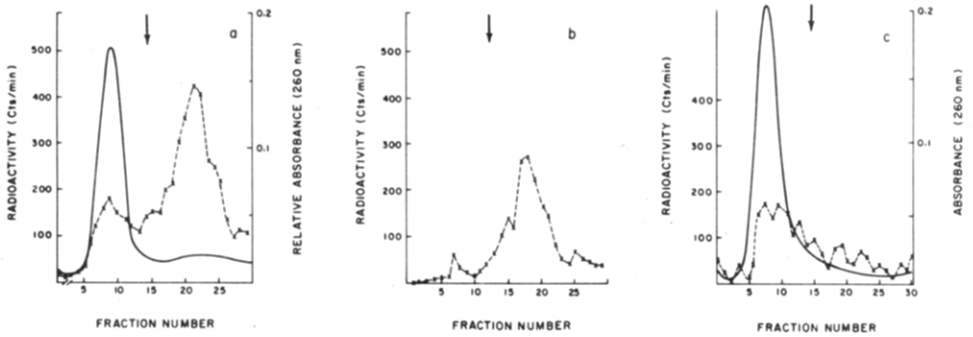

FIGURE 8: Hybridization of l-strand cRNA to sedimented mouse DNAs after *Eco*RI digestion. (a) Total mouse DNA (50 μg), (b) purified mouse satellite DNA (3 μg), and (c) purified main-band DNA (50 μg) were digested to completion with *Eco*RI and sedimented in a 10–40% sucrose gradient for 18 h at 20000 rpm in an SW 41 rotor. The DNA in each fraction was denatured, adsorbed to nitrocellulose filters, and hybridized with ^{3}H-labeled satellite cRNA. Absorbance at 260 nm (—); radioactivity (×---×). The arrow indicates the position of λ-DNA obtained from parallel gradients run in identical fashion.

folate reductase genes, appear to have concomitantly amplified closely associated satellite sequences (Bostock & Clark, 1980). Marker chromosomes contain extensive regions of c-banding material, similar to that found in mouse centromeric heterochromatin. After in situ hybridization with ^{125}I-labeled satellite DNA, silver grains are found over multiple noncentromeric sites of marker chromosomes that also apparently contain the amplified folate reductase genes.

The data in this report indicate that satellite sequences are, in fact, interspersed with nonsatellite DNA in the main band. The main-band-associated satellite sequences were identified by filter hybridzation with a labeled cRNA prepared from purified mouse satellite l strand to DNAs fractionated by isopycnic CsCl centrifugation. Main-band sequences that hybridize with the cRNA must have substantial homology with the major (A + T)-rich satellite since hybridization was performed under relatively stringent conditions and was followed by ribonuclease treatment. Although filter hybridization is useful for localization of satellite sequences within a gradient, it is insufficient for quantitating the percentage of satellite sequences that appear in main-band DNA. The level of hybridization to filter-bound DNA in the satellite region of the gradient is lower than expected. Because the major satellite sequences become highly concentrated in the gradient, it is likely that they reassociate before filtration and are thus unavailable for hybridization with the labeled cRNA probe, despite appropriate precautions described under Materials and Methods. Estimates of the percentage of satellite sequences represented in main-band DNA would therefore be inflated.

The possibility that the satellite-like sequences in the main band represent trapped contaminant derived from the satellite peak was addressed by mixing purified labeled satellite DNA with total unlabeled DNA and centrifuging to equilibrium in neutral CsCl. The radioactivity remained confined to the satellite region of the gradient (Figure 3), indicating that under the conditions used, physical trapping does not contribute to the occurrence of satellite sequences in the main band. The T_m of hybrids formed between satellite cRNA and DNAs from different regions of the gradient was similar, indicating that hybridization was to (A + T)-rich satellite-like DNA sequences in the main band as well as in the satellite-containing shoulder. This result is confirmed by the similarity in distribution of radioactivity in 2′,3′-mononucleotides produced by alkaline hydrolysis of satellite cRNA hybridized to DNA in all regions of the gradient.

The function of satellite DNAs is unclear. They generally reside in heterochromatin and appear not to be transcribed (Flamm et al., 1969), although sporadic reports claim to have detected transcripts that are complementary to satellite sequences (Harel et al., 1968; Cohen et al., 1973). The most convincing demonstration of satellite transcription is based on in situ hybridization of labeled satellite DNA to RNA associated with lampbrush chromosomes in amphibian oocytes (Varley et al., 1980). The reports claiming detection of mouse satellite transcripts may in fact have identified transcripts originating not from the satellite proper but from interspersed satellite DNAs. A highly repeated sequence has been identified as part of an intron in a cloned mouse albumin gene, and a homologous sequence is located distal to the 5′ side of a cloned mouse α-fetal protein gene (S. Tilghman, unpublished experiments). Whether these sequences have homology with the major satellite DNA is unknown, but the possibility is intriguing and is consistent with the reported association of satellite DNA and amplified dihydrofolate reductase genes (Bostock & Clark, 1980).

A provocative observation regarding the behavior of purified satellite DNAs has been reported by Skinner & Chambers (1977), who have shown that separated strands from a variety of satellites can form complexes with nonhomologous DNA [see Skinner (1977) for further discussion]. The phenomenon is not universal since not all satellites (e.g., human satellite I) or separated strands (e.g., l strand but not h strand of mouse satellite) form such associations. The physiological role of such associations, if any, is not known, but it is tempting to speculate that they may participate in introducing satellite sequences into nonsatellite regions of the genome to generate the interspersion described in the paper.

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