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Dispersity of Repeat DNA Sequences in *Oncopeltus fasciatus*, an Organism with Diffuse Centromeres

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Abstract. DNA of *Oncopeltus fasciatus*, an organism with diffuse centromeres, has been characterized by determination of its base composition, buoyant density, thermal stability, and reassociation kinetics; renatured DNA was characterized similarly. We conclude that repeated sequences are primarily short and scattered throughout the genome. This is in contrast to the extensive tandem repeats which are found in DNAs of organisms with discrete centromeres.

Introduction

Highly repetitive DNA is characteristically localized in centric heterochromatin in organisms ranging from insects to mammals (Yunis and Yasmineh, 1969; Hennig *et al.*, 1970; Jones, 1970; Jones and Robertson, 1970; Pardue and Gall, 1970; Rae, 1970; Arrighi *et al.*, 1971; Moore and Abuelo, 1971). These observations have led to speculation that the function of such repetitive sequences involves chromosome structure rather than the specification of gene products (Walker, 1971). Indeed, the simple nucleotide sequences of mouse, guinea pig, and *Drosophila* satellite DNAs make unlikely their encoding of amino acid sequence information (Southern, 1970; Gall, 1973). This is in accord with the absence, *in vivo*, of RNA complementary to mouse satellite DNA (Flamm *et al.*, 1969).

A common feature of chromosomes of many of the organisms mentioned above is the presence of discrete centromeres that appear to function as spindle-attachment sites during meiosis and mitosis. Chromosomes of some organisms, however, contain diffuse centromeres. These chromosomes are cytologically observed to be holokinetic (Wilson, 1912). We have isolated and characterized DNA from the milkweed bug, *Oncopeltus fasciatus* (Dallas), a hemipterous insect with diffuse centromeres (Wolfe and John, 1965; Comings and Okada, 1972). The arrangement of the fast-renaturing sequences ($C_0t \leqq 10^{-2}$ M-sec) of this DNA was of particular interest: are they arranged in tandem to give high molecular weight regions such as in the mouse (Kit, 1961; Pardue and

Gall, 1970) and *Drosophila* density satellite DNAs (Gall *et al.*, 1971), or, alternatively, are they distributed throughout the DNA paralleling the diffuse centromere regions?

Our results support the latter possibility. We found by buoyant density analyses, renaturation kinetics, thermal stability measurements, and by characterization with nuclease S_1 (Sutton, 1971) that less than one percent of *Oncopeltus* DNA is organized as high molecular weight tandemly repeated sequences. We infer that the fast-renaturing sequences are short and are widely spaced throughout the *Oncopeltus* genome.

Materials and Methods

1. Preparation of DNAs

Oncopeltus DNA was prepared by a modification of the methods of Marmur (1961) and Paul and Gilmour (1968). In a typical preparation, 10 g of 140—144 hr old eggs (Harris and Forrest, 1967), either fresh or stored at −70° C, were dechorionated by placing them in 5% sodium hypochlorite for five min, washed with about three liters of distilled water, and gently disrupted with a teflon–glass homogenizer in 150 ml 0.075 *M* NaCl – 0.030 *M* EDTA, pH 7.5, at 0° C. The homogenate was filtered through 400-mesh nylon screen to remove chorions and large fragments and then centrifuged at 1935 × g for 10 min. The pellet was suspended in 100 ml 0.1 *M* Tris-HCl – 0.15 *M* NaCl, pH 8, and lysed by adding sodium dodecyl sulfate (20% w/v) to a final concentration of 2%. After stirring 15 min at 20° C, the mixture was adjusted to 2 *M* NaCl; stirring was continued for 10 min. The resulting mixture was deproteinized with chloroform:2-octanol (24:1, v/v). Two volumes of ethanol were then layered on the aqueous phase, and the DNA was spooled out and redissolved in 5—10 ml 0.1 × SSC (1 × SSC is 0.15 *M* NaCl – 0.15 *M* trisodium citrate). The resulting solution was adjusted to 1 × SSC, digested for 30 min at 37° C with 10 units RNase T_1 (Schwarz/Mann) plus 10 μg pancreatic RNase/ml (Mann Research Laboratories, RNase A, heated at 80° C in 0.15 *M* NaCl, pH 5.0, for 10 min), and then digested an additional 2 hrs at 37° C with 50 μg pronase/ml (Calbiochem, autodigested). The mixture was repeatedly deproteinized with chloroform:2-octanol, and the DNA was finally spooled out of the aqueous phase after addition of two volumes of ethanol. The DNA was dissolved in either 0.1 × SSC or 0.02 *M* sodium phosphate buffer, pH 6.8 (PB), and stored at −20° C. Yields ranged from 700—1000 μg DNA/gram of eggs.

Bacillus subtilis DNA (strain W23, obtained from M. D. Chilton, University of Washington, Seattle) was labeled with thymidine-2-^{14}C and isolated by Marmur's procedure (1961). Thymidine-2-^{14}C-labeled DNA from bacteriophage T4 (grown on *Escherichia coli* B) was isolated by Freifelder's procedure (1968). *Micrococcus lysodeikticus* DNA (Miles Laboratory, Inc.) and *Myxococcus xanthus* DNA (a generous gift of J. Johnson) were repurified by ethanol precipitation prior to use; phage 2C DNA (*B. subtilis* host) was kindly provided by Dr. M. Mandel (M. D. Anderson Hospital and Tumor Institute, Houston, Texas). *Drosophila melanogaster* DNA was isolated from embryos according to Dickson *et al.* (1971). Calf thymus DNA (Type I, Sigma) was repurified before use according to Marmur's procedure (1961).

The purity and concentration of DNA solutions were determined by their absorption spectra (an absorbance of 1.0 at 260 nm was taken to indicate 47 μg/ml), diphenylamine reaction (Burton, 1956) and thermal denaturation behavior (Mandel and Marmur, 1968).

DNA samples sheared at 12000 psi were obtained using a French-type pressure cell (French and Milner, 1955) fitted with a steel ball valve; samples sheared at pressures other than 12000 psi were obtained using an Aminco pressure cell fitted with a nylon ball valve. Unless otherwise specified native *Oncopeltus* DNA was sheared twice at 14000 psi using the latter cell.

2. Chemical Determination of Base Ratios

DNA samples were hydrolyzed by heating with 90% formic acid at 170° C for 30 min or with 70% $HClO_4$ at 100° C for 35 min (Günther and Prusoff, 1967). The resultant bases were separated by descending chromatography on acid-washed Whatman No. 3MM paper using isopropanol: concentrated HCl: H_2O (65:16.7: H_2O to make 100 ml) as solvent system. The bases were located by their ultraviolet absorption, the spots were excised, and the bases were eluted with 0.1 *N* HCl and estimated spectrally.

3. Hydroxyapatite Fractionation of Denatured and Renatured DNA

Samples of sheared DNAs (50—200 μg) were fractionated into denatured and renatured components by hydroxyapatite chromatography (Bernardi, 1969; Laird, 1971) at 60° C unless otherwise indicated. Denatured DNA was eluted by 0.12 *M* PB and the renatured fraction by 0.5 *M* PB. Hydroxyapatite (Hypatite C, Clarkson Chemical Co., Inc.) columns (1×1—2 cm) were cycled with 0.001 *M*, 0.12 *M*, and 0.50 *M* PB and then equilibrated with 0.12 *M* PB prior to use. All DNA samples were adjusted to 0.12 *M* PB before application on the columns; usually six 2-ml fractions of both 0.12 *M* and 0.5 *M* PB eluates were collected.

4. Reassociation Kinetics as Measured by Hydroxyapatite Chromatography

The reassociation kinetics of sheared, heat-denatured *Oncopeltus* DNA was measured in PB by hydroxyapatite chromatography according to Britten and Kohne (1968). Sheared radiolabeled *B. subtilis* or T4 DNA was used as an internal standard (Laird, 1971). The concentration of the DNAs and of the PB used are included in the figure captions; the PB concentration was monitored by its refractive index. Reassociation was allowed to proceed at 60° C, usually in 0.12 *M* PB; when a more concentrated buffer solution was used to accelerate the renaturation, the rate of reassociation was corrected to that of the standard condition, 0.12 *M* PB, using the appropriate salt correction factor (Britten and Smith, 1970). Samples containing *ca.* 100 μg *Oncopeltus* DNA and 5—10 μg radiolabeled DNA were sealed in capillary tubing or in screw-cap vials closed with teflon tape, heat-denatured in a boiling water bath for 20 min, and allowed to renature at 60° C. Aliquots (individual sealed tubes) were removed at various times, quick-cooled in an acetone–dry ice bath, and fractionated by hydroxyapatite chromatography. The relative percent of denatured and renatured DNA present was determined by spectrophotometry and/or by measuring the radioactivity of the fractions. To measure the radioactivity of each fraction, bovine serum albumin (BSA; 200 μg) was added followed by aqueous trichloroacetic acid (TCA; 10% final concentration); the precipitate was collected, after keeping 1 hr at 0° C, on membrane filters (Bac-T-Flex, Type B-6, Schleicher and Schüll Co.); the filters were air-dried and counted in 10 ml toluene containing PPO (4 g/l) and POPOP (50 mg/l) using a Beckman LS-250 scintillation spectrometer (80% counting efficiency for ^{14}C). The percent of DNA renatured at time t was plotted versus the equivalent C_0t value, the product of the DNA concentration in molarity of nucleotides and time in seconds (Britten and Kohne, 1968); $C_0t_{1/2}$ is the C_0t value corresponding to 50% renaturation.

5. Buoyant Density Determinations in Neutral CsCl

DNA samples were centrifuged to density equilibrium in CsCl (Schwarz/Mann, optical grade) gradients at either 42040 rpm using 2° sector Kel-F centerpieces in an An-F rotor or 40000 rpm using 2° double-sector centerpieces in an An-G rotor. A Spinco Model E analytical ultracentrifuge with UV photographic attachment was used for the former runs and a Model E with multiplexer scanner for the latter. On-scale representation of both a marker DNA (1 μg) and a DNA present in a large excess was achieved by scanning at different wavelengths. Buoyant densities were determined relative to *Micrococcus lysodeikticus* DNA (ϱ=1.731 g/cm³), *E. coli* DNA (ϱ = 1.710 g/cm³), *Myxococcus xanthus* DNA (ϱ = 1.727 g/cm³), or phage 2C DNA (*B. subtilis* host, ϱ=1.742 g/cm³) included as an internal density marker. The guanine plus cytosine content (G+C) was calculated according to Mandel *et al.* (1968).

6. Determination of Molecular Size by Sedimentation Analysis

The double- and single-strand molecular weights of DNA samples were determined by velocity sedimentation at 42040 rpm under neutral (1 *M* NaCl — 0.01 *M* Tris-HCl, pH 8.0) and alkaline (0.1 *M* NaOH — 0.9 *M* NaCl) conditions, respectively, using a band-forming centerpiece (Spinco No. 331346), An-D rotor, and a Spinco Model E analytical ultracentrifuge (Studier, 1965). Strand lengths were calculated assuming 330 daltons per nucleotide unit.

7. Analytical Ultracentrifugation of DNA in Ag^{+}- and Hg^{2+}-Cs_2SO_4 Density Gradients

Mercuric and silver ions bind preferentially to AT-rich and GC-rich regions of DNA, respectively, thus increasing the density of the DNA. Solutions of DNAs (*ca.* 5 μg), repurified Cs_2SO_4 (Szybalski, 1968; ϱ=1.53 g/cm³ based on the refractive index), and various concentrations of $AgNO_3$ (Corneo *et al.*, 1971) or $HgCl_2$ (Nandi *et al.*, 1965) in 0.005 *M* borate buffer, pH 9.2, were centrifuged 20 hrs at 42040 rpm using 2° sector Kel-F centerpieces, an An-F rotor, and a Spinco Model E analytical ultracentrifuge.

8. Reversible Thermal Dissociation (Hyperchromic Measurement)

The change in optical density of DNAs in 0.1×SSC at 260 nm as a function of temperature was followed continuously using a Gilford 2400 spectrophotometer. The temperature corresponding to the midpoint of the hyperchromic shift (T_m) and the corresponding G+C values were calculated according to Mandel and Marmur (1968). Values are corrected relative to the T_m of *B. subtilis* DNA (71.4° C in 0.1×SSC) which was determined simultaneously. T_m values of some samples were also obtained by plotting the percent absorbancy increase at 260 nm versus temperature on normal probability paper (Knittel *et al.*, 1968).

9. Irreversible Thermal Dissociation (from Hydroxyapatite)

The midpoint of the irreversible thermal dissociation ($T_{m,i}$) of DNA was determined analogously to the T_m value except that the DNA was absorbed on a hydroxyapatite column and the elution profile was measured as a function of temperature. A solution of sheared native *Oncopeltus* DNA (*ca.* 120 μg) and ^{14}C-labeled *B. subtilis* DNA (720 or 1040 dpm/μg; 1—5 μg as internal standard) in 0.12 *M* PB was passed through a hydroxyapatite column at 60° C and the column was washed free of non-adsorbed DNA. The adsorbed DNA was sequentially

thermally eluted in 5° increments from the ethylene glycol-jacketed column using preheated 0.12 *M* PB. The final fraction was obtained by elution with 0.5 *M* PB at 60° C. The DNA content of each fraction was determined by spectrophotometry and by scintillation spectrometry of the precipitated DNA as described above. Base compositions were estimated using as a standard the $T_{m,i}$ value determined for *B. subtilis* DNA (42.6% G+C), *i.e.* 91.5° C (Mandel and Marmur, 1968). The change in optical density of the DNA eluted by 0.12 *M* PB as a function of temperature was small (7—12%), gradual, and occurred largely below 50° C, which is consistent with the absence of double-stranded DNA in eluates derived from native DNA and from DNA-DNA duplexes. Thus, we conclude that our denaturation of DNA from hydroxyapatite occurred within the acceptable range of PB concentrations (Martinson, 1972).

10. Preparation and Assay of Nuclease S_1

Nuclease S_1 was prepared from *Aspergillus oryzae* (Sanzyme, Calbiochem) according to Sutton's method (1971). After DEAE-cellulose chromatography, fractions showing better than 8-fold purification over the dialyzed crude extract were pooled, divided into aliquots, and stored at −70° C without apparent loss of enzyme activity in 6 months. The enzyme was assayed by a modification of Sutton's procedure in which the incubation mixture contained 0.03 *M* sodium acetate buffer, pH 4.5, 3×10^{-5} *M* $ZnSO_4$, 0.01 *M* NaCl, 2.9 μg sheared, heat-denatured ^{14}C-labeled *B. subtilis* DNA (1680 dpm/μg), 25 μg sheared, heat-denatured calf-thymus DNA, and 0.2—10 μl of nuclease S_1 preparation in a final volume of 0.3 ml. Following incubation of the mixture at 50° C for 10 min, 0.2 ml of cold 0.2% (w/v) BSA and 0.5 ml cold 20% TCA were added, and the mixture was then cooled in an ice bath for 10 min, and centrifuged to obtain the acid-soluble supernatant. BSA and TCA were added to control samples at zero time. The radioactivity present in a 0.5 ml aliquot of the supernatant was measured in 10 ml toluene containing PPO (4 g/l), POPOP (50 mg/l), and 10% BBS-3 (Beckman Instruments) using a Beckman LS-250 scintillation spectrometer. Expressing total radioactivity in the acid-soluble fraction as a percentage of the total substrate radioactivity, one unit of nuclease S_1 was defined under these conditions as the amount releasing 1% of the radioactivity from single-stranded ^{14}C-labeled *B. subtilis* DNA into the supernatant fraction in 10 min. The specific activity of nuclease S_1 refers to units per μg of enzyme protein. When sheared native DNA was incubated with nuclease S_1 under the same experimental conditions no release of DNA into the acid-soluble fraction was detectable.

11. Isolation of Fast-renaturing Oncopeltus DNA

Native, sheared *Oncopeltus* DNA (1.7—2.0 mg/3 ml) in 0.12 *M* PB was heat-denatured in a boiling water bath for 20 min, quick-cooled in an ice-salt bath ($C_0t \leqq 10^{-2}$ *M*-sec), warmed to 60° C and immediately chromatographed on hydroxyapatite (2 ml bed volume). The reassociated fraction, eluted with 0.5 *M* PB, was adjusted to 0.12 *M* PB and rechromatographed on a fresh hydroxyapatite column; the fractions eluted by 0.5 *M* PB (>90% of the recycled DNA) represented 10—15% of the total DNA. Appropriate fractions were pooled, dialyzed against 1000 volumes of 0.02 *M* NaCl or 0.1×SSC, and stored at −20° C.

12. Preparation of Nuclease S_1-treated Fast-renaturing Oncopeltus DNA

A fast-renaturing fraction of *Oncopeltus* DNA (about 10—15% of the total DNA) was digested with nuclease S_1 (10 μl, specific activity 90 units/μg protein) for 10 min at 50° C in a mixture containing 200—300 μg DNA in 0.01 *M* NaCl,

0.03 *M* sodium acetate buffer, pH 4.5, and 3×10^{-5} *M* $ZnSO_4$ in a final volume of 0.3 ml. The reaction mixture was then quick-cooled and diluted with 1 ml cold 0.12 *M* PB. After adjusting the PB concentration to 0.12 *M* on the basis of its refractive index, the mixture was fractionated by hydroxyapatite chromatography. The nuclease S_1-digestible DNA (58% of the total) was eluted with 0.12 *M* PB, whereas the nuclease S_1-resistant fraction (42%) was eluted with 0.5 *M* PB. The nuclease S_1-resistant fractions from several replicates were pooled, dialyzed against 0.02 *M* NaCl, adjusted to 1 *M* NaCl, and ethanol precipitated. The precipitate, which was dissolved in 0.02 *M* NaCl or 0.1 × SSC, was designated as the nuclease S_1-resistant component of the fast-renaturing fraction. The digested fraction, eluted with 0.12 *M* PB, showed no detectable hyperchromicity at 260 nm during thermal denaturation.

Results and Discussion

1. General Characteristics of Oncopeltus DNA

The base composition of *Oncopeltus fasciatus* DNA is about 32% G+C as determined by chemical analysis (Table 1). No unusual bases were detected. Buoyant density measurements in neutral CsCl density gradients indicate that the DNA distribution is unimodal with a mean density of 1.694 g/cm³ (Fig. 1a, b) which corresponds to 34% G+C. When neutral CsCl density gradients containing a large excess of either crude egg lysate or purified total DNA were centrifuged to equilibrium a very small band, representing 0.1—0.2% of the total DNA, with a buoyant density of 1.712 g/cm³ was observed (Fig. 1d, e). This small band may correspond to a previously reported satellite (Musich, 1971; Musich, personal communication). It may represent ribosomal DNA since the amount and base composition (53% G+C) correspond to those expected for ribosomal DNA.

Thermal elution of sheared *Oncopeltus* DNA from hydroxyapatite gave a midpoint of irreversible dissociation ($T_{m,\,i}$) of 87.5° C, while *B. subtilis* DNA, which served as an internal control, exhibited a $T_{m,\,i}$ of 91.5° C (Fig. 2A); these data indicate that *Oncopeltus* DNA contains 33% G+C. The reversible thermal dissociation of *Oncopeltus* DNA, followed optically by measuring the change in hyperchromicity at 260 nm, gave a T_m of 65.6° C in 0.1 × SSC (Fig. 2B) indicating 28% G+C; the hyperchromic shift was approximately 39%. These data are summarized in Table 1. The first three methods yield values for the base composition which are comparable, *i.e.* about 33% G+C; the value of 28% G+C calculated from the T_m, however, is markedly lower. Although discrepancies between the estimates of the base composition by different methods are usually indicative of the presence of unusual bases (Mandel and Marmur, 1968), no unusual bases were observed. This discrepancy may be indicative of an unusual structural feature of the DNA.

Table 1. Physical properties and base distribution of total native *Oncopeltus* DNA[a, b]

Buoyant density[c]		Thermal stability						Base ratios, mole-%[d]				
		Reversible dissociation (optical)			Irreversible dissociation (hydroxyapatite)							
					Oncopeltus		*B. subtilis*					
ϱ, g/cm³	% G+C	T_m, °C	% G+C	Hyperchromicity, %	$T_{m,i}$, °C	% G+C	$T_{m,i}$, °C	A	T	G	C	(G+C)
1.6944	35.1*	65.7	28.8	40.3	87	33	91.5	33.0	33.0	16.8	16.8	33.6*
1.6936	34.3	65.7	28.8*	39.2	88	33	92	34.7	33.6	16.7	14.0	30.7*
1.6934	34.1	65.4	28.1	37.9	87	33	91	33.5	34.8	14.8	17.0	31.8*
1.6934	34.1	65.4	28.1*	38.0				33.8	34.2	15.2	16.2	31.4
1.6939	34.6											
1.6933	33.9											
1.6937 ±0.0003	34.1 ±0.5	65.6 ±0.01_5	28.4_5 ±0.3_5	38.8 ±0.9	87.5	33	91.5	33.8 ±0.5	34.2 ±0.6	15.9 ±0.9	16.0 ±1.0	31.9 ±0.9

[a] Values were obtained using DNA isolated from 140—144 hr old embryos or 5th instar nymphs (designated with an asterisk).
[b] Experimental details for determination of the buoyant density, thermal stabilities, and base composition are given in Materials and Methods (p. 350).
[c] Values determined in CsCl using internal density marker DNAs: *Myxococcus xanthus* DNA ($\varrho = 1.727$ g/cm³), *Bacillus subtilis* DNA ($\varrho = 1.703$ g/cm³; 2nd entry), or *Micrococcus lysodeikticus* DNA ($\varrho = 1.731$ g/cm³; 6th entry).
[d] Chemically determined base ratios following hydrolysis by 90% HCO_2H (first three entries) or 70% $HClO_4$ (last entry); details are given in Materials and Methods (p. 350). Averages of triplicate determinations are given.

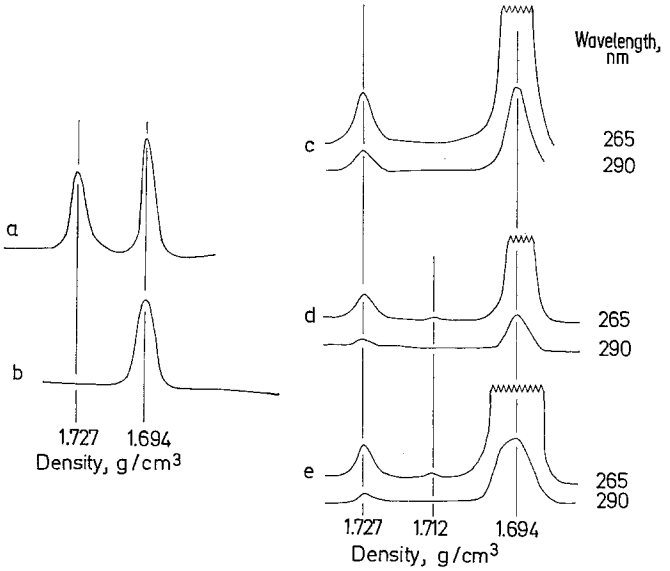

Fig. 1. Buoyant density profiles of various unsheared *Oncopeltus* DNA preparations determined in neutral CsCl with and without *Myxococcus xanthus* DNA (1 μg, $\varrho = 1.727$ g/cm³) as an internal density marker. Samples *a* and *b* were centrifuged at 42040 rpm, samples *c*—*e* at 40000 rpm. (*a*, *b*) Total native DNA (ca. 3 μg), (*c*) total native DNA (ca. 10 μg), (*d*) crude embryo (140—144 hr) lysate (Dickson *et al.*, 1971); 10 μg of DNA estimated to be present by on-scale representation. (*e*) Same as *c* except that 17 μg of DNA was estimated to be present. The small peak at 1.712 g/cm³ represents 0.1—0.2% of the total DNA

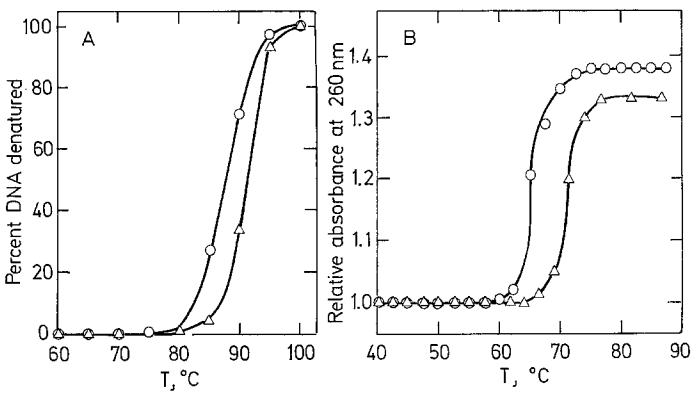

Fig. 2. (A) Irreversible thermal denaturation profiles of native, sheared (12000 psi) *Oncopeltus* (○, 100 μg) and ^{14}C-labeled *B. subtilis* DNAs (△, 1 μg, 1040 dpm/μg). (B) Thermal denaturation profiles of native, unsheared *Oncopeltus* (○) and *B. subtilis* DNAs (△) measured spectrophotometrically in 0.1 × SSC

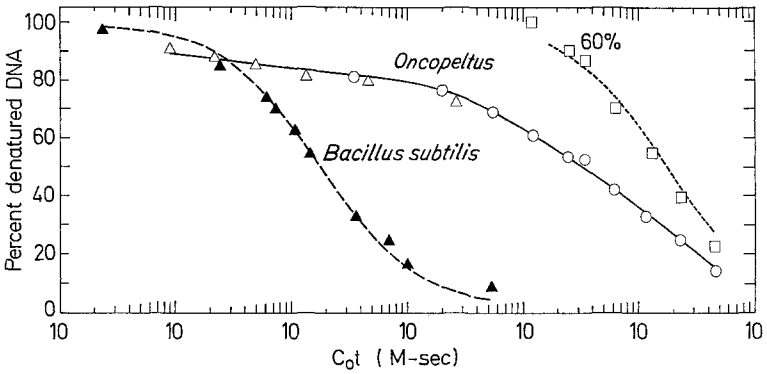

Fig. 3. Reassociation kinetics of sheared (12000 psi) total *Oncopeltus* and *B. subtilis* DNAs determined by hydroxyapatite chromatography. The DNAs were heat-denatured and allowed to renature in 0.12 *M* PB at 60° C; aliquots were taken over a period of 400 hrs. The *Oncopeltus* DNA concentrations were (μg/ml): △, 47; ○, 1150. ^{14}C-labeled *B. subtilis* DNA (▲, 13 μg/ml, 1040 dpm/μg), which renatures with 2nd-order kinetics, was present in the reaction mixtures as an internal standard. The dashed line represents the theoretical 2nd-order kinetic curve which fits the *Oncopeltus* data assuming 60% unique sequences

To determine the sequence diversity of the *Oncopeltus* genome, the reassociation kinetics of the DNA fragments were measured using hydroxyapatite chromatography (Britten and Kohne, 1968). The DNA fragments used in these studies had an average double-strand size of 809 nucleotide pairs and a single-strand size of 709 nucleotides (Studier, 1965), based on a molecular weight of 330 daltons per nucleotide. *Oncopeltus* DNA exhibits polyphasic renaturation kinetics (Fig. 3). Curve fitting of the renaturation kinetic data (Laird and McCarthy, 1969) from *Oncopeltus* suggests that about 60% of the DNA consists of unique sequences and 40% of repeated sequences. The 60% which exhibited second-order kinetics, depicted by the dashed line in Fig. 3, renatured about 1000 times slower than *B. subtilis* DNA. A correction for G+C difference between the DNAs reduced this ratio to about 855 (Wetmur and Davidson, 1968). According to the theoretical and empirical relationships between haploid genome size and $C_0t_{1/2}$ values (Laird, 1971), these data would imply a unique sequence genome of 1.7×10^{12} daltons ($855 \times 2 \times 10^9$ daltons). Since this portion represents 60% of *Oncopeltus* DNA, the haploid genome should consist of 2.8×10^{12} daltons (1.7×10^{12} daltons/0.6). About 10% of *Oncopeltus* DNA renatured at a $C_0t \leqq 10^{-1}$ *M*-sec. This fraction, designated as fast-renaturing DNA, was isolated and partially characterized (see below).

Separation of *Oncopeltus* DNA into fractions containing fast- and slow-renaturing sequences was attempted by allowing the renaturation

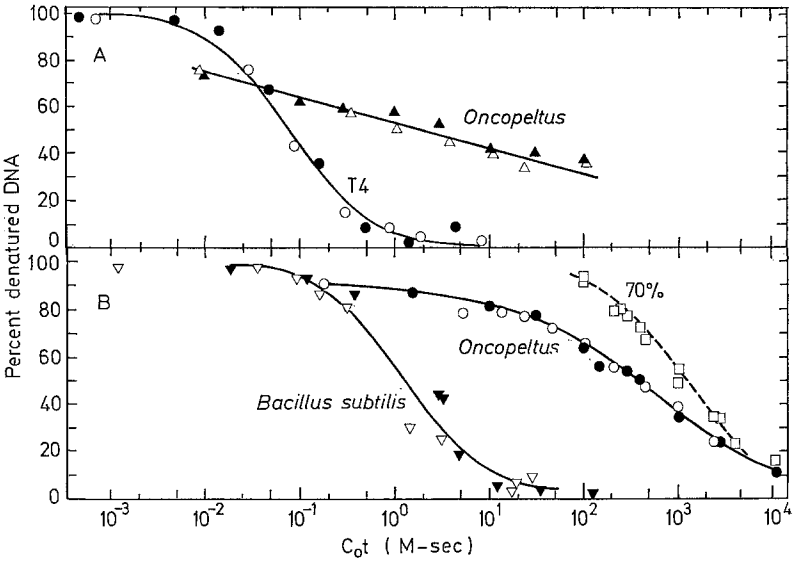

Fig. 4 A and B. Reassociation kinetics of fractionated *Oncopeltus* DNA as determined by hydroxyapatite chromatography. Sheared total *Oncopeltus* DNA in 0.12 *M* PB was heat-denatured, allowed to renature to a C_0t value of 120 *M*-sec at 60° C, and fractionated by hydroxyapatite chromatography. Appropriate fractions were pooled, dialyzed against 0.02 *M* NaCl, and, after adjusting to 1 *M* NaCl, the DNA was precipitated with ethanol from the aqueous phase and resuspended in phosphate buffer. After addition of a radiolabeled DNA to serve as an internal standard, the mixtures were heat-denatured, allowed to renature at 60° C, and aliquots fractionated by hydroxyapatite chromatography. The results of two independent experiments are summarized in the figure.

(A) Renaturation of repeated sequences of *Oncopeltus* DNA (38.6% of total DNA which was renatured by C_0t of 120 *M*-sec) in the presence of ^{14}C-labeled T4 DNA (1060 dpm/μg) in 0.12 *M* PB. Final DNA concentrations were (μg/ml): repeated *Oncopeltus* DNA, 99.4 (△), 110.8 (▲); ^{14}C-labeled T4 DNA, 8.0 (○), 5.2 (●)

(B) Renaturation of sequences of *Oncopeltus* DNA which had not renatured by C_0t of 120 *M*-sec (61.4% of total DNA) in the presence of ^{14}C-labeled *B. subtilis* DNA (651 dpm/μg). Final DNA concentrations were (μg/ml): *Oncopeltus* DNA, 2020 (○), 1350 (●); ^{14}C-labeled *B. subtilis* DNA, 14.0 (▲), 16.3 (△). The renaturation experiment designated by open symbols was conducted in 0.12 *M* PB, that designated by closed symbols was conducted in 0.577 *M* PB and corrected to 0.12 *M* PB according to Britten and Smith (1970). The dashed line represents the theoretical 2nd-order kinetic curve which fits the *Oncopeltus* data assuming 70% non-repeated sequences and corresponds to 43% of the total DNA

reaction to proceed to a C_0t value of 120 *M*-sec which corresponded to about 40% renaturation (cf. Fig. 3). The renatured and unrenatured fractions were separated by hydroxyapatite chromatography, and the renaturation kinetics of these two fractions were measured (Fig. 4).

The rapidly renaturing fraction, which represented 39% of total DNA, is 50% renatured ($C_0t_{1/2}$) at a C_0t value of 2 and apparently consists of a very complex group of related families (Fig. 4A). As expected, the rapidly renaturing fraction contains a larger proportion of the fast-renaturing DNA, *i.e.* renaturation was about 25% complete by a C_0t of 10^{-2} M-sec. In contrast, the 61% fraction, which had not renatured by a C_0t of 120 M-sec, exhibited a $C_0t_{1/2}$ of 370 M-sec upon subsequent renaturation (Fig. 4B). The kinetic data do not follow a second-order relationship indicating that the fraction still contained some repeated sequences. Curve fitting of these data suggest that 70% of this fraction (cf. dashed line in Fig. 4B), or 43% (70% × 61%) of the total DNA, consisted of unique sequences which renatured with a $C_0t_{1/2}$ of 1200 M-sec. This value is 920 times greater than the $C_0t_{1/2}$ value of the *B. subtilis* control (Fig. 4B). A correction for G+C content similar to that discussed above would give a unique sequence genome of 1.6×10^{12} daltons ($785 \times 2 \times 10^9$ daltons), or a total genome of 3.7×10^{12} daltons (1.6×10^{12} daltons/0.43).

2. Specific Tests for High Molecular Weight Repeat Sequences

The presence of very rapidly renaturing *Oncopeltus* DNA fragments is suggestive of the existence of repeat sequences in this organism. Since these fragments renature so rapidly that kinetics are difficult to measure optically, specific tests for high molecular weight repeat sequences were made.

Oncopeltus DNA remained unimodal when centrifuged to equilibrium in Cs_2SO_4 density gradients containing either silver or mercuric ions. Density satellites were not detected at a variety of Hg^{2+} and Ag^{+} concentrations (Fig. 5a–c). In control experiments, the density satellites present in *Drosophila melanogaster* DNA (Blumenfeld and Forrest, 1971) were resolved (Fig. 5d). Identical results were obtained using *Oncopeltus* DNA fragments of about 800 nucleotide pairs; *i.e.*, no discrete density satellites were observed.

Enrichment of the rapidly renaturing fraction by hydroxyapatite chromatography and subsequent cesium chloride pycnography were performed to assay for a small fraction of high molecular weight repeats. The fast-renaturing *Oncopeltus* DNA (18% of total) appeared as a single band with a very small less-dense shoulder (of about native density, 1.694 g/cm^3) when centrifuged to density equilibrium in neutral CsCl gradients (Fig. 6A, c); the broad band width is indicative of duplexes of low molecular weight and/or heterogeneous density. The buoyant density ($\varrho = 1.709$ g/cm^3) of this fraction is considerably higher than that observed for native DNA ($\varrho = 1.694$ g/cm^3) indicating re-establish-

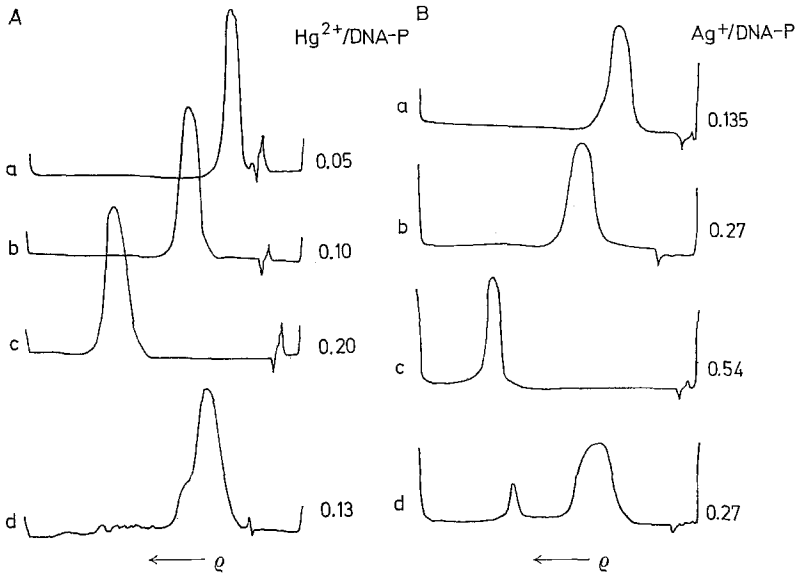

Fig. 5A and B. The distribution of total native, unsheared *Oncopeltus* DNA in (A) Hg^{2+}-Cs_2SO_4 and (B) Ag^{+}-Cs_2SO_4 equilibrium density gradients at several metal ion/DNA-P concentrations (tracings *a*, *b*, and *c*). *Drosophila* DNA, which contains density satellites, was used as control (tracing *d*; total, native unsheared DNA)

ment of only a small portion (ca. 20%; see legend to Fig. 8) of the base pairs. Cesium chloride pycnography of more extensively renatured DNA (78% renatured; Fig. 6A, d) showed a greater return to native density (ca. 50%, $\varrho=1.703$); there was no indication of a bimodal density distribution of the DNA.

The experiment illustrated in Fig. 6A, c, a neutral CsCl gradient of 18% fast-renaturing DNA, shows a small shoulder at the density of native DNA. Musich (personal communication) has observed a peak in this position when analyzing, by density, the fast-renaturing DNA which binds at 0.2 *M* PB, but elutes at 0.3 *M*. From Musich's data, we infer that this fraction comprises about 5% of the total DNA; of this 5%, about 13—14% appears to have native-like density. Thus of the total DNA, about 0.7% seems to band at native density after renaturation. We have analyzed by our methods five independently prepared DNAs for this fast-renaturing fraction and arrive at a similar value for the proportion of total DNA which renatures to a native-like density. In our experiment, we analyzed the renatured fractions eluting between 0.12 and 0.5 *M* PB. Neutral CsCl pycnography of these renatured DNAs (Fig. 6B, a–e) shows variable amounts of DNA in this

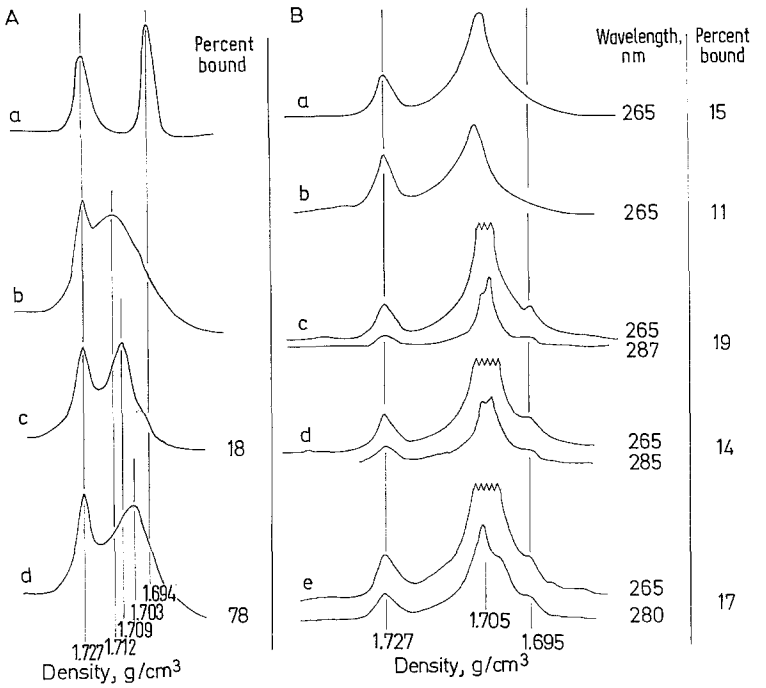

Fig. 6A and B. Buoyant densities of reassociated *Oncopeltus* DNA isolated by hydroxyapatite chromatography after partial renaturation. Sheared (A; 30000 psi), heat-denatured DNA was allowed to renature in 0.12 *M* PB at 60° C. Samples were allowed to renature partially and were then fractionated. That portion of the DNA which was adsorbed on hydroxyapatite in 0.12 *M* PB and eluted with 0.5 *M* PB was subjected to CsCl equilibrium density-gradient centrifugation (Mandel *et al.*, 1968). Each sample contained *Myxococcus xanthus* DNA (ϱ= 1.727 g/cm^3) as a density marker

(A) Tracings of (*a*) unfractionated, native, unsheared DNA, (*b*) unfractionated, heat-denatured, sheared DNA, (*c*) 18% of total DNA, renatured by a C_0t value of 4.95×10^0 *M*-sec, and (*d*) 78% of total DNA, renatured by a C_0t value of 7.00×10^3 *M*-sec

(B) Tracings *a*—*e* represent the fast-renaturing fraction (11—19% of total DNA) isolated from five independent DNA preparations. When excess DNA was used (*c*—*e*), a small peak at 1.695 g/cm^3 was observed. This peak was estimated to represent about 5% of the DNA present (0.7% of total DNA) in tracing *c*

region, presumably because of differences in band broadness caused by differences in the molecular weight of the DNA. The largest shoulder in the native region (Fig. 6B, c) comprised about 5% of the renatured fraction, or 0.7% of the total DNA, in close agreement with the value observed by Musich. We do not know if this represents palindrome sequences which self renature, cross-linked strands that did not separate

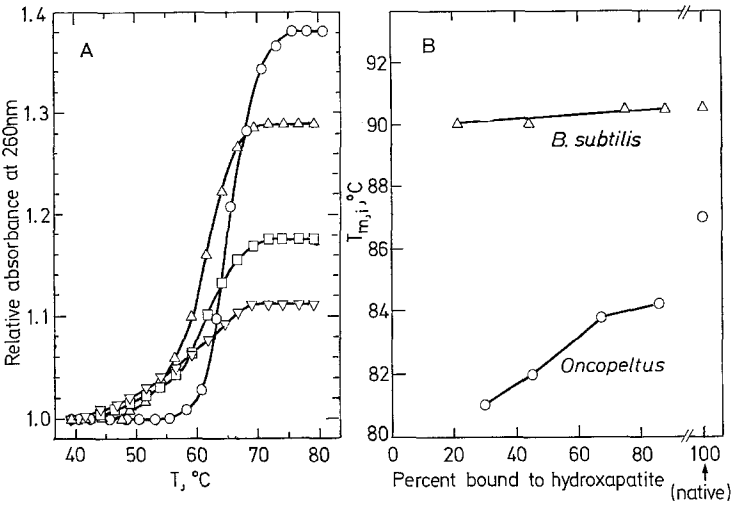

Fig. 7. (A) Thermal denaturation of reassociated *Oncopeltus* DNA after varying extents of renaturation: ▽, 48.1%; □, 70.3%; and △, 86.7% renatured. Sheared (12000 psi) heat-denatured DNA was allowed to renature at 60° C and then chromatographed on hydroxyapatite. The duplexes adsorbed in 0.12 *M* PB and eluted with 0.5 *M* PB were dialyzed against 0.1×SSC and their thermal stabilities determined optically. Analogous data for native, sheared, total DNA (○) are included for comparison

(B) Irreversible thermal stabilities of DNA/DNA duplexes isolated by hydroxyapatite chromatography after various times of incubation at 60° C in 0.12 *M* PB. This experiment was done in combination with that shown in Fig. 3. The $T_{m,i}$ values of aliquots containing partially renatured *Oncopeltus* and ^{14}C-labeled *B. subtilis* DNAs (1040 dpm/μg) were determined as described in Materials and Methods. The $T_{m,i}$ values of native sheared, total *Oncopeltus* and ^{14}C-labeled *B. subtilis* DNAs are included for comparison

upon denaturation, or repeats of tandem organization. In any case, this fraction comprises less than one percent of the total DNA.

The thermal stabilities, both reversible (Fig. 7A) and irreversible (Fig. 7B), of various renatured fragments of *Oncopeltus* DNA were measured to test the suggestion that the early renaturing fragments are substantially unpaired. The percent hyperchromicity observed on reversible thermal denaturation of the reassociated fragments increased with longer times of renaturation; after 48% renaturation, the hyperchromicity of renatured DNA was about 11%; after 87% renaturation the hyperchromicity had increased to about 29%. Sheared native DNA exhibited an optical hyperchromicity of 38%. The T_m values of the various reassociated fractions remained essentially constant at 61° C (in 0.1×SSC), as compared to 65.6° C for native *Oncopeltus* DNA. The

irreversible thermal dissociation of the reassociated DNA fragments indicated a slight increase in the stability of the duplexes isolated between 30% and 87% reassociation; the $T_{m,\,i}$ values increased from 81° C to about 84° C compared with a $T_{m,\,i}$ value of 87° C for native *Oncopeltus* DNA. In contrast, *B. subtilis* DNA/DNA duplexes isolated between 20% and 88% renaturation exhibited an essentially constant $T_{m,\,i}$ of 90—90.5° C, in agreement with the stability of native *B. subtilis* DNA.

The extent of renaturation of fragments binding to hydroxyapatite indicates a concordance between the buoyant density (open circles) and the hyperchromic shift accompanying reversible thermal denaturation (closed circles) (Fig. 8). Thus we conclude that these fragments contain less than one percent rapidly renaturing DNA which exhibits extensive re-establishment of base pairing.

3. Effect of Fragment Size on the Amount of Fast-renaturing DNA

The observation that fast-renaturing *Oncopeltus* DNA fragments exhibit only limited base-pairing is consistent with long unrenatured stretches separated by short well-paired regions, or with unpaired bases distributed randomly throughout reassociated fragments. These alternatives were tested by renaturing fragments of differing size. *Oncopeltus* DNA, ranging from a mean of 1450 nucleotide pairs to a mean of 600 nucleotide pairs, was allowed to renature to $C_0t \approx 10^{-2}$ M-sec, and the percentage renatured DNA was determined by hydroxyapatite chromatography. The percentage of renatured *Oncopeltus* DNA decreased from about 11% to 3.5% as the fragment size decreased, whereas the amount of renatured ^{14}C-labeled *B. subtilis* DNA, which served as internal standard, increased slightly (1.5% to 3.2%) (Fig. 9). These data support the suggestion that the slower-renaturing sequences in *Oncopeltus* DNA are adjacent to short, rapidly renaturing sequences and indicate that the rapidly renaturing sequences can be separated from the slowly renaturing regions by extensive shearing. Similar conclusions were presented for *Drosophila melanogaster* DNA from euchromatic regions (Wu *et al.*, 1972).

4. Characterization of the Fast-renaturing Fraction

Although the fast-renaturing fraction of *Oncopeltus* DNA would appear to be substantially unpaired (cf. Figs. 6 and 8), the well-paired regions of such fractions can be isolated using a nuclease which preferentially hydrolyzes single-stranded DNA (Brahic and Fraser, 1971; Sutton, 1971). The fast-renaturing fraction of *Oncopeltus* DNA was obtained by allowing the renaturation reaction to proceed to a C_0t

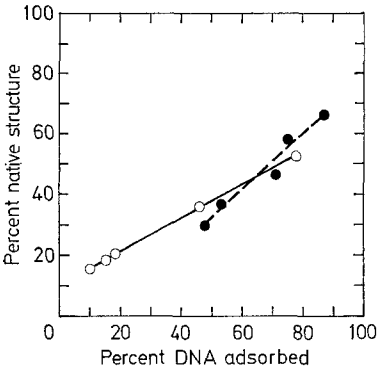

Fig. 8

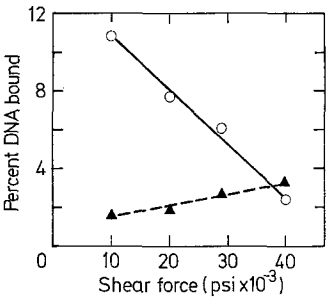

Fig. 9

Fig. 8. Percent native structure of *Oncopeltus* DNA fragments bound to hydroxyapatite after various extents of renaturation. The proportion of native-like structure of the duplexes was taken from the percent return to the "native" (ϱ= 1.694 g/cm^3) from the "denatured" (ϱ=1.712 g/cm^3) buoyant density (○) and was also determined from the proportion of the hyperchromicity of the reassociated DNA relative to that of native DNA (●)

Fig. 9. The relationship between shear force and the percent fast-renaturing *Oncopeltus* DNA (○; $C_0t \leqq 10^{-2}$ *M*-sec) retained by hydroxyapatite. ^{14}C-labeled *B. subtilis* DNA (▲) served as an internal standard. Portions of a mixture of *Oncopeltus* DNA (94 μg/ml) and ^{14}C-labeled *B. subtilis* DNA (2 μg/ml; 1040 dpm/μg) in 0.12 *M* PB were sheared; aliquots (1 ml) of each sample were denatured by heating at 100 °C for 20 min and immediately fractionated by hydroxyapatite chromatography

value of about 1×10^{-2} *M*-sec and isolating the renatured fraction (10—15% of total) by hydroxyapatite chromatography. This fraction was digested with nuclease S_1 (Sutton, 1971), and the nuclease-resistant fraction, 42% (4—6% of total; 42% × 10—15%) of the fast-renaturing DNA, was isolated by hydroxyapatite chromatography. The 58% removed by digestion showed no observable hyperchromic shift at 260 nm, and was not further characterized. Nuclease S_1 had no measurable digestive activity toward total native *Oncopeltus* DNA.

The nuclease S_1-resistant component of the fast-renaturing fraction and the fast-renaturing fraction itself were characterized analogously to total *Oncopeltus* DNA (cf. Table 1) except that the small amount of material available precluded chemical determination of the base ratios. Analytical velocity centrifugation under neutral conditions (Studier, 1965) indicated that the untreated fraction sedimented with a $S^0_{20,w}$ value of 9.9 whereas the nuclease S_1-resistant component sedimented with a value of 6.5 (Table 2). Diffusion, due to the small sizes of these DNA fragments, prevented accurate determination of molecular weights

Table 2. Molecular sizes of the fast-renaturing fraction of *Oncopeltus* DNA before and after treatment with nuclease S_1[a]

DNA	Velocity sedimentation			Electron microscopy[b]		
	$S^{\circ}_{20,w}$	daltons	nucleotides per strand	μ	daltons	nucleotides per strand
Fast-renaturing fraction ($C_0t \leqq 10^{-2}$ M-sec)						
1) nuclease S_1-resistant	6.5	2.6×10^5	390[c]	0.16	3.2×10^5	450
2) untreated[d]	9.9	5.9×10^5[e]	—	(0.23)	—	—
Total, sheared						
1) neutral conditions	8.4	5.3×10^5	810	0.28	5.6×10^5	820
2) alkaline conditions	7.4	2.3×10^5	710	—	—	—

[a] For derivation of DNA samples, and experimental details, see Materials and Methods (p. 350).
[b] Preliminary data obtained using the method of Davis *et al.* (1971).
[c] Calculated assuming perfect pairing.
[d] Only experimental values are given since the presence of extensive single-stranded regions precludes use of standard constants to calculate molecular weight.
[e] Molecular weight estimated using the relationship $S^{\circ}_{20,w} = 9.9 = 42\%\ 0.0882\,M^{0.346} + 58\%\ 0.0528\,M^{0.4}$, which is based on Studier's equations (1965) and assumes 42% of the untreated fraction is well-paired.

using analytical sedimentation techniques. However, assuming perfect pairing and using Studier's constants (Studier, 1965; footnote e, Table 2), the nuclease S_1-resistant fraction had a molecular weight of approximately 2.6×10^5 daltons, which is about half the size of the sheared native DNA (5×10^5 daltons) used in these experiments. These values are in good agreement with the sizes estimated by electron microscopy (preliminary data included in Table 2). The size of the untreated fast-renaturing fraction was calculated to be about 5.9×10^5 daltons based on the observation that 42% of the untreated fragments were nuclease S_1-resistant and were assumed to be well-paired. On the basis of these data, the untreated fragments do not appear to be appreciably aggregated.

The nuclease S_1-resistant component of the fast-renaturing fraction sedimented more slowly than yeast tRNA (4S) in a neutral sucrose gradient, whereas the native sheared DNA sedimented slightly faster (Fig. 10). If yeast tRNA is taken as a standard to calculate relative sizes, the nuclease S_1-resistant fraction is about half the size of sheared native *Oncopeltus* DNA (3.6 S $\approx$ 100 base pairs and 4.8 S $\approx$ 230 base

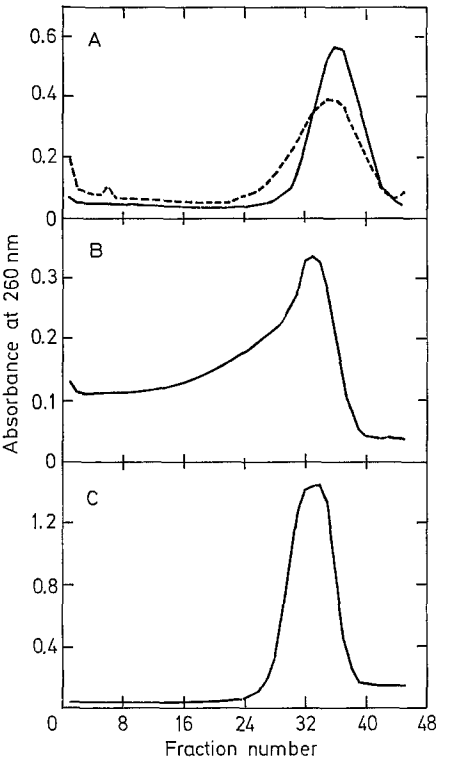

Fig. 10A—C. Sedimentation of (A) the nuclease S_1-resistant component of the fast-renaturing fraction of *Oncopeltus* DNA (——, ca. 140 μg) and an external yeast tRNA marker (– – – –, ca. 30 μg, Mann Research Laboratories), (B) untreated fast-renaturing fraction (ca. 470 μg), and (C) sheared, native *Oncopeltus* DNA (ca. 520 μg) in linear 5—20% neutral sucrose density gradients (0.05 *M* Tris-HCl, 0.2 *M* NaCl, pH 7.0). Centrifugation was carried out for 17 hrs at 25000 rpm and 4° C in a Spinco Model L ultracentrifuge with a SW-25 rotor. The gradient was dripped from the bottom and the absorbancy (260 nm) was read for each 550 μl fraction

pairs, respectively). Presumably the absolute size estimate is different from that obtained using Studier's method because of the difficulties in using an RNA as a sedimentation standard. The distribution of untreated fast-renaturing DNA fragments was much broader than that of either the nuclease S_1-resistant fraction or native sheared DNA, indicating markedly greater size heterogeneity, although the position of the peak fraction corresponded with that of native sheared DNA (Fig. 10C). Comparison of the sedimentation patterns of the treated and untreated fast-renaturing fractions indicates that nuclease S_1 treat-

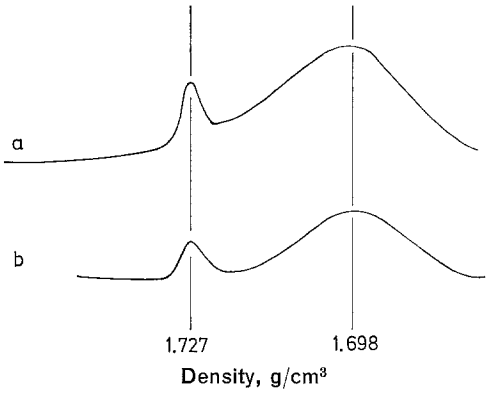

Fig. 11. Buoyant density profiles of (*a*) the nuclease S_1-resistant component of the fast-renaturing fraction and (*b*) total native, sheared *Oncopeltus* DNA determined in neutral CsCl with *Myxococcus xanthus* DNA (ϱ=1.727 g/cm³) as an internal density marker

ment resulted in more homogeneous size distribution, presumably by the digestion of unpaired regions.

Attempts to determine the buoyant density of the nuclease S_1-resistant fraction by CsCl isopycnography yielded a single, very broad, symmetrical band centered about ϱ=1.70 g/cm³; diffusion due to the small fragment size precluded precise determination of the peak position (Fig. 11a). Sheared native DNA also had a higher than expected density of 1.698 (Fig. 11b). In contrast, the untreated fast-renaturing fraction banded as an unsymmetrical peak at about ϱ=1.705 g/cm³ with small less-dense shoulders comprising <1% of total DNA (Fig. 6B, c–e). The presence of unpaired regions in the untreated fraction makes it more dense than the nuclease S_1-resistant fraction.

As shown in Fig. 12 and Table 3, the hyperchromicity of the fast-renaturing fraction increased from 22 to 36% following nuclease S_1 treatment indicating an increase in the relative amount of base pairing in the nuclease-resistant component. The T_m value of the nuclease-resistant fraction was 61° C in 0.1×SSC as compared with 66° C for the total native DNA. The melting profile of the nuclease-resistant component was broader than that of the total sheared native DNA, which could be a reflection of a high degree of heterogeneity in the base composition, the small fragment size, and/or some imperfect pairing. These data were plotted on probability paper (Knittel *et al.*, 1968); total DNA gave a straight line (Fig. 12B) indicating that the base composition is predominantly homogeneous throughout the DNA. Data for the nuclease-resistant component (Fig. 12B) also yield a straight

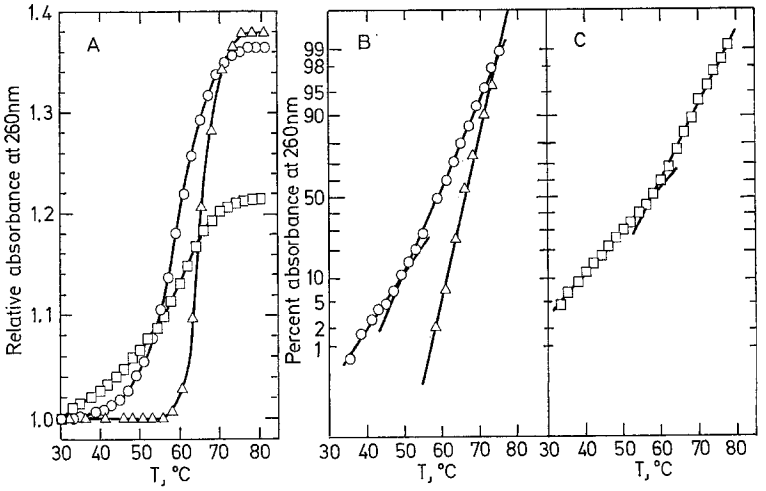

Fig. 12A—C. Linear (A) and probability (B and C) graphs of thermal denaturation of the fast-renaturing fraction of *Oncopeltus* DNA ($C_0t \leqq 10^{-2}$ *M*-sec) before (□) and after (○) treatment with nuclease S_1 plotted using values determined spectrophotometrically in 0.1×SSC. The corresponding data for native, sheared, total DNA (△) are included for comparison

Table 3. Thermostability of the fast-renaturing fraction of *Oncopeltus* DNA before and after treatment with nuclease S_1[a]

DNA	Reversible dissociation			Irreversible dissociation	
	T_m, °C	% G+C	Hyperchromicity, %	$T_{m,i}$, °C	% G+C
Fast-renaturing fraction ($C_0t \leqq 10^{-2}$ *M*-sec)					
1) nuclease S_1-resistant	61.2	17.8[b]	35.9	84.8	23.4[b]
2) untreated[c]	58.5	—	22.3	84.0	—
Total, native *Oncopeltus*	65.6	28.5	37.6	89.1	33.7
Bacillus subtilis	71.4	42.6	38.0	92.8	42.6

[a] For derivation of DNA samples, and experimental details, see Materials and Methods (p. 350).

[b] Calculated assuming perfect pairing and that Mandel and Marmur's (1968) relationship holds below 30% G+C.

[c] Only experimental values are given since the presence of extensive single-stranded regions precludes calculation of the base composition.

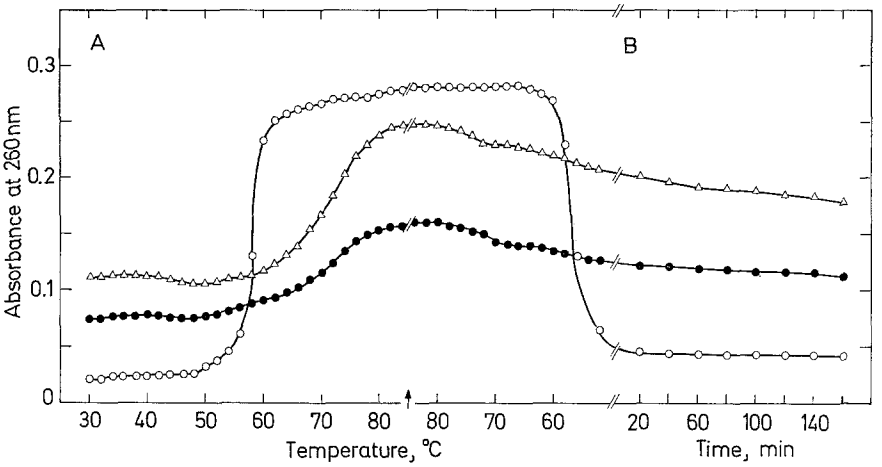

Fig. 13A and B. Denaturation and renaturation profiles, measured spectrophotometrically, of d(A-T) (○) and the fast-renaturing fraction of *Oncopeltus* DNA before (●) and after (△) treatment with nuclease S_1. The DNA samples were denatured in 0.5 *M* PB at the following concentrations (absorbance at 260 nm given): d(A-T), 0.435; untreated, 0.245; and nuclease S_1-treated, 0.250. The hyperchromicity accompanying denaturation as the temperature was increased from 30 to 85° C is illustrated in part A; data points between 85 and 93° C are not shown but are denoted by the arrow and the discontinuities in the curves. The cuvette temperatures were then lowered from 93 to 50° C to allow either snap-back of single strands containing inverted repeats (palindrome sequences) or renaturation of complementary strands to occur. With d(A-T), almost complete hypochromicity occurred within several degrees of the T_m (58° C). *Oncopeltus* DNA, however, showed a gradual hypochromicity which continued as the cuvette temperature was maintained at 50° C (part B)

line if points below 47° C are ignored; T_m values reported for A-T rich DNAs are >47° C in 0.1 × SSC (Davidson *et al.*, 1965; Blumenfeld and Forrest, 1971). The lower temperature points in Fig. 12B and C, representing about 10% (0.3—0.5% of total DNA) and 50% (5—7% of total DNA) components of the samples, respectively, probably correspond to random unfolding of the DNA, not strand separation. The line connecting the data points above 50° C for the nuclease-resistant component clearly lies to the left of the line for total DNA suggesting that the nuclease-resistant component of the fast-renaturing fraction is more A-T rich than total DNA, although this conclusion is not consistent with the buoyant density in CsCl (Fig. 11a). Comparison of Fig. 12B and C shows that the amount of random unfolding is reduced, but not completely eliminated, by nuclease S_1 treatment.

Irreversible thermal dissociation of the nuclease-resistant component gives a $T_{m,\,i}$ value of 84.8° C as compared with a value of 89.1° C for total DNA (Table 3), again suggesting that the former is more A-T rich than the latter.

To determine whether the fast-renaturing DNA was a result of hairpins due to palindrome sequences (Wilson *et al.*, 1972), the hypochromicity during reassociation was compared with that of a palindromic DNA. d(A-T) was used for this control, as it forms hairpin self-renatured molecules at a temperature only a few degrees below its T_m. During cooling of the fast-renaturing DNA (untreated and nuclease S_1-resistant fractions), the hypochromicity decreased slowly with temperature (and time), as opposed to the dramatic hypochromicity of d(A-T) within a few degrees of its T_m (Fig. 13). Thus, the renaturation of the nuclease S_1-resistant fraction appears to result from association of complementary strands rather than from intrastrand renaturation or snap-back caused by cross-linking between the strands.

5. Concluding Remarks

Oncopeltus DNA which has been sheared into fragments of about 800 nucleotide pairs exhibits polyphasic renaturation kinetics: (1) 10—15% of the DNA is fast-renaturing and probably contains repeat sequences, (2) about 45% consists of a complex group of related families and renatures at an intermediate rate, and (3) the remainder, *ca.* 45%, is composed of unique sequences. The haploid genome size is estimated to be 2.8 to 3.7×10^{12} daltons based on renaturation kinetics. Native DNA bands in CsCl at $\varrho = 1.694$ g/cm^3 which corresponds to 34% G+C, in agreement with the base composition determined chemically and by thermal denaturation. When a large excess of DNA is submitted to CsCl isopycnography a small band, representing less than 0.2% of the total DNA, can be detected at 1.712 g/cm^3. The amount and base composition suggest that this peak could represent ribosomal DNA. No unusual bases were observed.

The fast-renaturing sequences purified on hydroxyapatite are only about 40% base-paired and are not appreciably aggregated. This was inferred from comparison of the thermal stability, and from the size characterization by velocity sedimentation of the fast-renaturing fragments before and after digestion with nuclease S_1 to remove unpaired regions. These findings, together with the fact that the amount of fast-renaturing DNA can be reduced by decreasing the fragment size, are interpreted as evidence that the renatured DNA sequences are short (400 nucleotides) and non-contiguous.

We predict from these results that repeat sequences in *Oncopeltus* DNA are widely scattered throughout the genome, in contrast to the

high concentration near the centromere of repeat DNA sequences in *Drosophila* (Gall *et al.*, 1971) and mouse (Jones and Robertson, 1970; Pardue and Gall, 1970). This interpretation correlates with the diffuse centromeres of *Oncopeltus* chromosomes which confer holokinetic activity (Hughes-Schrader and Ris, 1941), presumably as a consequence of spindle fiber attachment sites that are widely spaced throughout the chromosomes (Comings and Okada, 1972). The lack of discrete centromeres in *Oncopeltus* may place limits on the biological tolerance of the extent of tandemly organized repeat sequences.

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