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Characterization of the Genome of the Free-Living Nematode *Panagrellus silusiae*: Absence of Short Period Interspersion†

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ABSTRACT: The complexity of the DNA of the free-living nematode *Panagrellus silusiae* has been examined. Reassociation kinetics of pressure-sheared fragments (~290 nucleotides) in 0.18 M Na^+ at 60 °C showed the presence of foldback, repetitive, and unique DNA sequence elements. The three classes comprise 9.3%, 26.1%, and 61.3% of the total DNA, respectively. The mean length of the foldback duplex DNA after digestion with S_1 nuclease is about 185 nucleotides. There are about 1.8×10^4 inverted repeats per genome. Sequence arrangement was deduced from (1) renaturation kinetic profiles of long and short fragments on hydroxylapatite; (2) the pattern of renaturation of tracer DNA, labeled in vitro with ^{125}I, of various sizes after incubation with excess short fragments; and (3) thermal denaturation behavior of DNA that had been reassociated to various C_0t values. It was found that DNA fragments of the repetitive fraction that are, at least, 2000 nucleotides in length are virtually free of unique sequences. Moreover, it is estimated that the repeated segments in this species could extend for 10000 nucleotide pairs. Thus, *Panagrellus* DNA lacks the pattern of extensive short period interspersion that is typified by the DNA of *Xenopus*.

The genomes of all eukaryotes that have been characterized to date contain foldback, repetitive, and single copy DNA sequence elements. In most cases, much of the repetitive DNA is intimately interspersed with unique DNA sequences. In *Xenopus laevis*, for example, over 50% of the genome comprises middle repetitive elements that are about 300 nucleotides long and are interspersed with single copy sequences that are about 1000 nucleotides in length (Davidson et al., 1973). This dominant pattern has been designated as "short period interspersion" (Davidson et al., 1975). In various organisms, the extent of the short period interspersed DNA ranges from 50% to 80% of the total genome (Britten et al., 1976). By contrast, the genomes of three insect species (*Drosophila melanogaster*, Manning et al., 1975; Crain et al., 1976a; *Apis mellifera*, Crain et al., 1976b; *Chironomus tentans*, Wells et al., 1976), the water mold (*Achlya ambisexualis*, Hudspeth et al., 1977), and two vertebrates (chicken, Arthur & Straus, 1978; Syrian hamster, Moyzis et al., 1977) are known to lack, for the most part, short interspersed sequences.

It has been estimated that in *Drosophila* DNA the repetitive elements are localized in tracts about 5600 nucleotides long and the single copy sequences occur in segments of 13000 nucleotides or more. The functional aspects of DNA sequence organization are not known; however, Britten and Davidson have suggested that the interspersed repeated elements of the "short period" pattern are coordinating regulators for contiguous structural genes (Britten & Davidson, 1969; Davidson et al., 1977). Although the absence of the short period interspersion in some animals would seem to negate this particular theory of gene regulation, it has been suggested that a long period pattern could have evolved from a short period pattern by selective deletion of specific DNA segments (Crain et al., 1976b; Davidson et al., 1977).

Panagrellus silusiae, a free-living nematode, has one of the smallest genomes (0.09 pg) that has been reported for a multicellular eukaryote (Pasternak & Haight, 1975). In addition to its small genome size, *Panagrellus* and other free-living nematodes have some significant biological attributes. For example, about nine somatic cell divisions are required during embryogenesis to form a fully differentiated multicellular organism (Sin & Pasternak, 1970; Deppe et al., 1978). Increasingly, the nematode is being used as an experimental system for studying the mechanisms of aging, behavior, and cellular differentiation (e.g., Brenner, 1974; Gershon, 1970; Hirsh & Vanderslice, 1976; Leushner & Pasternak, 1975; Hedgecock & Russell, 1975; Harris et al., 1977). Therefore, it is of intrinsic interest to examine the organization of the DNA of such an organism.

In the present study we examined the DNA reassociation

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kinetics of *Panagrellus silusiae* and found that the DNA sequences of this nematode are not arranged in the short period "Xenopus" pattern but rather the repeated sequences of *P. silusiae* are clustered in the genome.

Materials and Methods

Culture Methods. The free-living nematode *Panagrellus silusiae* was grown on bacteria at 25 °C on 4.5% (w/v) Czapek Dox agar (Samoiloff & Pasternak, 1969). Worms were floated free of contaminating organisms by several passages through a Baermann filtration apparatus. The worms were either used immediately or stored at −196 °C. For some experiments, *P. silusiae* was grown axenically in a modified version of Rothstein's medium (Rothstein, 1974). The medium contains 40 g of soy peptone, 10 g of yeast extract, 30 mg of β-sitosterol, 10 mg of cholesterol, and 10 mg of ergosterol per L of distilled water to which was added 500 mg of sterile-filtered bovine hemoglobin per L.

DNA Extraction. DNA extraction was carried out by a slight modification of the "MUP" technique (Britten et al., 1974). Nematodes (10–20 mL wet-packed volume) were resuspended in 8 M urea and 0.24 M phosphate buffer (equimolar mixture of the NaH_2PO_4 and Na_2HPO_4, pH 6.8) and broken open in a Braun MSK cell homogenizer by shaking with an equal volume of glass beads (0.5 mm diameter, microbeads) at 4 °C. The homogenate was filtered through a stainless steel screen (50 × 50 mesh), brought to 1 M sodium perchlorate and 1% sodium dodecyl sulfate in a total volume of 100 mL. After mixing the mixture for 2 min at low speed in a Sorval Omnimixer, 0.5 volume (50 mL) of chloroform:isoamyl alcohol (24:1) was added and the mixing was resumed at maximum speed for 2 min. The emulsion was spun at 3000*g* for 10 min. The aqueous phase was removed and mixed with hydroxylapatite (4 g, Bio-Gel, HTP, Bio-Rad). The slurry was filtered through a triacetate filter (0.22 mm pore size, 47 mm diameter; Gelman). The hydroxylapatite from the filter was suspended into successive 20-mL aliquots of 8 M urea in 0.24 M phosphate buffer until the absorbance at 260 nm of the filtrate was less than 0.1. The hydroxylapatite was washed with successive 10-mL aliquots of 0.012 M phosphate buffer until the urea was removed (as judged by refractometry). Native DNA was eluted from the hydroxylapatite with 0.5 M phosphate buffer. All DNA-containing fractions ($A_{260} > 0.5$) were pooled, dialyzed into 0.5 mM EDTA[1] (pH 8.0), and stored, over chloroform, at 4 °C.

The purity of the DNA samples was monitored by thermal denaturation in a Beckman DU monochromator equipped with a Gilford photometer and water-jacketed cuvette holder. During melts, the temperature was controlled manually and measured with a linear thermistor probe (Yellow Springs Instruments). All native DNA preparations exhibited hyperchromicity of about 0.27 when calculated from the formula $H = (A_{260}^{98°C} - A_{260}^{60°C})/A_{260}^{98°C}$, where H is hyperchromicity, $A_{260}^{98°C}$ is the absorbance (260 nm) at 98 °C, and $A_{260}^{60°C}$ is the absorbance (260 nm) at 60 °C. The A_{260}:A_{230} and A_{260}:A_{280} ratios of all DNA preparations were approximately 2.4 and 1.9, respectively.

In order to discount mitochondrial DNA as a significant contaminant of the DNA preparations from whole worms, DNA was extracted from a crude nuclear fraction. About 15 mL of wet-packed worms was suspended in 30 mL of buffer (0.25 M sucrose, 0.005 M magnesium acetate, 0.025 M KCl, and 0.05 M Tris, pH 7.5) and broken in a Braun homogenizer. The homogenate was spun at 100*g* for 15 min at 4 °C. Phase microscopy revealed that the pellet contained nuclei along with some contaminating pieces of cuticle. The pellet was washed repeatedly at low speeds before being suspended into 8 M urea, 0.24 M phosphate buffer, 1% sodium dodecyl sulfate, and 1 M sodium perchlorate. DNA was extracted as described above.

Shearing and Sizing of DNA Fragments. To obtain small DNA fragments, DNA in 0.5 mM EDTA was sheared by two passages through the needle valve of an Aminco French pressure cell at 30000 psi at a rate of 30 drops per min (Straus & Birnboim, 1974). Larger fragments were produced by passing DNA (250 μg/mL in 0.5 mM EDTA) through a 27-gauge syringe needle 20 times.

The sizes of the single-stranded DNA fragments were determined by band sedimentation in 0.9 M NaCl, 0.1 M NaOH at 40000 rpm and 20 °C in a Spinco Model E Ultracentrifuge (Studier, 1965). The mean size of the single-stranded fragments that had been sheared in the French pressure cell was 292 ± 42 nucleotides ($n = 4$). The mean size of unsheared or needle sheared DNA ranged from 1926 to 5928 nucleotides, before boiling, in different preparations.

DNA Reassociation. The reassociation procedures were essentially those of Britten et al. (1974). Prior to reassociation, DNA samples and phosphate buffers were passed over Chelex-100 resin (Bio-Rad). DNA (0.25–1000 μg/mL) samples were denatured in 0.12 M phosphate buffer in screw cap test tubes by immersion into boiling water until the sample temperature reached 98 °C. Thereafter, the tubes were maintained at 60 °C until the desired C_0t value was reached. A C_0t value is computed from the absorbance at 260 nm of the initial DNA sample at 98 °C times 0.5 multiplied by the period in hours during which the denatured DNA sample was held at 60 °C. Each data point was obtained by passing a 100-μg aliquot of DNA over 0.5 g of hydroxylapatite in a jacketed column maintained at 60 °C. Unreassociated DNA was eluted with 10 mL of 0.12 M phosphate buffer at 60 °C. Reassociated DNA was eluted with 10 mL of 0.12 M phosphate buffer at 98 °C. The fraction of the DNA remaining single stranded (i.e., C/C_0) at a particular C_0t value was calculated by taking the A_{260} of that eluant at 60 °C and dividing by the sum of A_{260} values that were eluted at 60 and 98 °C. Each A_{260} value was corrected for scatter due to hydroxylapatite by subtracting out a correction factor based on the absorbance at 320 nm.

S_1 Nuclease Digestion. S_1 nuclease (Miles Laboratories) digestion of reassociated DNA was conducted according to the procedure of Goldberg et al. (1975). A sample of DNA in 0.2 M NaCl, 0.07 M Pipes (Sigma) buffer, pH 6.7, was denatured at 100 °C and incubated to the desired C_0t value. After chilling, incubation buffer was added to a final concentration of 0.15 M NaCl, 0.05 M Pipes buffer, 0.025 M sodium acetate, and 0.1 mM $ZnSO_4$, pH 4.55. The enzyme was added to a concentration of 100 units per μg of DNA. The mixture was incubated for 45 min at 37 °C. These conditions simulate a "digestion estimate" of 0.95 as defined by Britten et al. (1976). The reaction was terminated by chilling and bringing the incubation mixture to 0.12 M with respect to phosphate buffer. Digested material and S_1 nuclease were separated from duplex DNA by binding the latter material to hydroxylapatite at 60 °C.

Iodination of Panagrellus DNA. Panagrellus DNA was iodinated in vitro with $Na^{125}I$ (New England Nuclear, carrier

[1] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); PPO, 2,5-diphenyloxazole; BBS-3, Biosolv, Beckman.

Table I: Kinetic Analysis of Pressure-Sheared *Panagrellus* DNA

component	genome fraction[a]	rate constant, K^b	$C_0t_{1/2}$	kinetic complexity	rel no. of copies	$C_0t_{1/2}$ (pure)
repetitive	0.261 ± 0.017	10.3 ± 3.6	0.0967	2.78×10^4	861	0.0252
single copy	0.613 ± 0.019	0.012 ± 0.002	83.3	5.62×10^7	1	51.0

[a] Zero-time fraction comprises 0.093 and unreassociated DNA makes up 0.033 ± 0.016 of the total DNA. [b] K in whole DNA, M^{-1} s^{-1}.

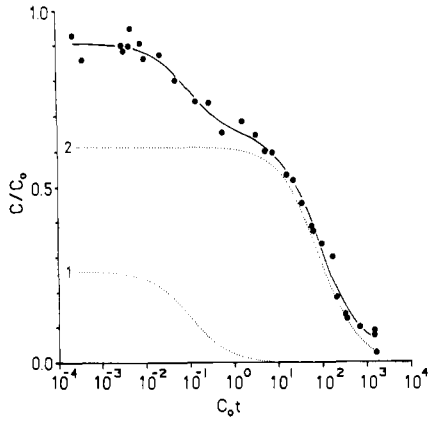

FIGURE 1: Reassociation kinetics of pressure-sheared *Panagrellus* DNA. The fraction of DNA fragments containing duplex regions after renaturation was estimated by hydroxylapatite binding. The solid curve represents a computer-generated best fit of the data with two-component, ideal, second-order curves (Kells & Straus, 1977). The dotted lines depict the solutions of the computer analysis for repetitive (1) and single copy (2) components separately.

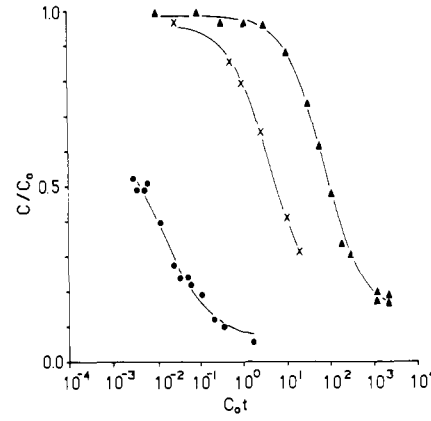

FIGURE 2: Reassociation kinetics of *Panagrellus* DNA fractionated on hydroxylapatite. Highly sheared DNA was heat denatured, incubated to a C_0t of 122, and passed over hydroxylapatite at 60 °C. Single-stranded DNA was eluted with 0.12 M phosphate buffer and double-stranded DNA with 0.5 M phosphate buffer. Double-stranded DNA was heat denatured, incubated to C_0t 0.1, and analyzed on hydroxylapatite. C_0t plots were determined with both sets of DNA. The $C_0t_{1/2}$(pure) for repetitive and single copy components were 8.68 $\times$ 10^{-3} and 56.3, respectively. The $C_0t_{1/2}$ for *E. coli* DNA was 3.8. Symbols: (▲) single copy DNA (C_0t 122, 28% unbound); (●) repetitive DNA (C_0t 122, 72% bound; C_0t 0.1, 31% bound); (X) internal standard, [^{3}H]DNA (9×10^4 cpm/μg) from *E. coli*.

free, low pH) by the method of Chan et al. (1976). Final specific activity of the DNA was about 4×10^5 cpm/μg.

Alkaline Sucrose Gradient Centrifugation. To isolate labeled DNA of various lengths, samples were layered onto a 4.8-mL 5–20% (w/v) linear sucrose gradient in 0.9 M NaCl, 0.1 M NaOH, and 1 mM EDTA. After 16 h at 25 000 rpm and 20 °C in a SW 50L, gradients were fractionated. Sedimentation coefficients for the labeled DNA fragments were calculated with respect to two unlabeled DNA standards included in each run (Burgi & Hershey, 1963).

Counting Methods. Labeled DNA samples from C_0t plots consisted of 5 mL of 0.12 M phosphate buffer that was mixed with 10 mL of PCS (Phase Combining System, Amersham/Searle) and counted. Other aqueous samples were counted in a toluene–PPO–BBS-3 mixture (1000 mL of toluene, 7 g of 2,5-diphenyloxazole, 100 mL of BBS-3 (Biosolv, Beckman)).

Results

A representative hydroxylapatite C_0t (in units of M s throughout paper) curve of the renaturation of pressure-sheared DNA fragments from *Panagrellus* is illustrated in Figure 1. The form of this plot indicates that the genome of *Panagrellus* consists of three kinetic components: (1) a foldback (zero-time) fraction that renatures with a C_0t value of less than 10^{-3}; (2) an intermediate reannealing fraction that renatures between $C_0t \sim 2 \times 10^{-3}$ and 10^0; and (3) a slowly renaturing component that reanneals between C_0t 10^0 and 10^3. Table I contains the computer-estimated rates of reaction and size of each of the components in Figure 1 (Kells & Straus, 1977). The $C_0t_{1/2}$ of the last component (83.3 M s) is 21.9 times larger than the $C_0t_{1/2}$ of *E. coli* (Figure 2). Since the genome size of *E. coli* is about 4.42×10^{-3} pg, the kinetically estimated genome size of *Panagrellus* is 0.097 pg. By comparison, a value of 0.09 pg per *Panagrellus* genome was obtained by Feulgen microspectrophotometry (Pasternak & Haight, 1975). This close agreement indicates that the slowly renaturing component is single copy DNA.

In order to characterize the repetitive and single copy DNA components further, the reassociation kinetics of each isolated fraction was examined (Figure 2). The purified single copy fraction is kinetically homogeneous with ~84% of the DNA reassociating with ideal second-order kinetics (triangles, Figure 2). The $C_0t_{1/2}$ pure value, 56.3, for this fractionated component is in good agreement with the $C_0t_{1/2}$ pure predicted in Table I. The isolated repetitive DNA component (circles, Figure 2), as expected, contains a substantial amount of DNA that has reassociated by C_0t 3×10^{-3}. The amount of this renatured DNA is approximately what one would predict from both the amount of foldback in total DNA (based on the amount of iodinated DNA that reacts by C_0t 10^{-6}) and the fact that the procedure used to isolate the repetitive DNA component enriches for foldback sequences. The reassociation kinetics for the remainder of the DNA that reacts over the range of C_0t 3×10^{-3} to C_0t 1 are consistent with the fit of a one-component, ideal, second-order curve with $C_0t_{1/2}$ pure value of 8.68×10^{-3}.

To test for the interspersion of repeated and unique sequences, the hydroxylapatite-monitored DNA reassociation profile of larger DNA fragments that were about 2000 nucleotides in length (Figure 3) was compared with the reassociation pattern of more highly sheared DNA (see Figure 1). Repetitive and single copy components comprised 27.9 ± 2.7% and 58.9 ± 1.5% of the total genome respectively after renaturation of the longer DNA fragments. If a significant fraction of the repeated elements is interspersed with unique elements one would expect a substantial increase in the estimated size of the repeated component due to the fact that

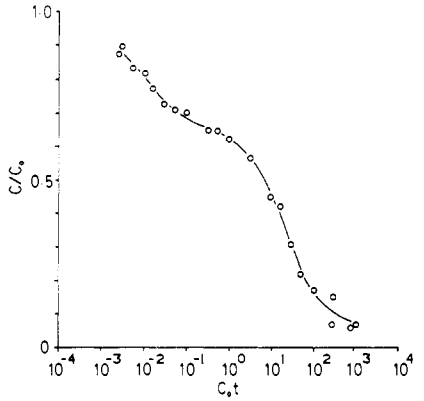

FIGURE 3: Reassociation kinetics of *Panagrellus* DNA on hydroxylapatite. DNA fragments of 2000 nucleotides (○) were reassociated in 0.12 M phosphate buffer at 60 °C.

unreassociated unique sequences would bind to hydroxylapatite because of their physical attachment to renatured repeated sequences. Comparison of the DNA reassociation profiles of Figures 1 and 3 shows that, although the length of the longer DNA fragments is 6.8 times that of the shorter DNA fragments, the amount of the material that corresponds to the repeated component has increased from 0.261 to 0.279 of the total genome. At this level of discrimination there appears to be very little interspersion of repeated and unique DNA sequences.

The apparent absence of short period interspersion may be due to a substantial contribution of mitochondrial DNA to the "repetitive fraction" of the extracted DNA from whole worms. The low complexity of mitochondrial DNA and its independence from nuclear DNA could account for the observations here. To eliminate this possibility, C_0t curves of purified nuclear DNA revealed no differences from the reassociation patterns with whole worm DNA. The repetitive fraction in nuclear DNA was 0.260 which agrees with the value of 0.261 for whole worm DNA.

Hyperchromicity measurements on DNA that is reassociated to different C_0t values can yield information about the sequence organization (e.g., Goldberg et al., 1975). Interspersion would be evident at low C_0t values because hydroxylapatite-bound DNA would be partially duplexed. The single copy DNA would remain unreassociated while the adjacent repetitive regions are base paired. As a consequence of partial duplex formation, hyperchromicity values, in comparison with native DNA, would be strikingly depressed. *Panagrellus* DNA that consisted of either small or large fragments was denatured, reassociated to a particular C_0t value, and thermally melted (Figure 4). The relative hyperchromicities of small and large DNA pieces at C_0t values of 0.5, 10, and 100 are listed in Table II. The extent of hyperchromicity did not vary appreciably as the C_0t values of the DNA fragments were increased. Short fragments at each C_0t value show slightly more duplex formation than the corresponding larger pieces. In a further analysis, large (2300 nucleotides) and small DNA fragments were both stripped of foldback DNA and incubated to C_0t 0.2. The melting parameters of these DNAs indicated that the relative hyperchromicity estimates for the small and large fragments were 0.918 and 0.826, respectively (Figure 5 and Table II). These data suggest that single copy DNA is seldom linked to repeats in fragments of, at least, 2000 nucleotides.

A tracer reassociation experiment provides another means for examining DNA sequence organization. Since the specific activity of in vivo labeled DNA from *Panagrellus* is low,

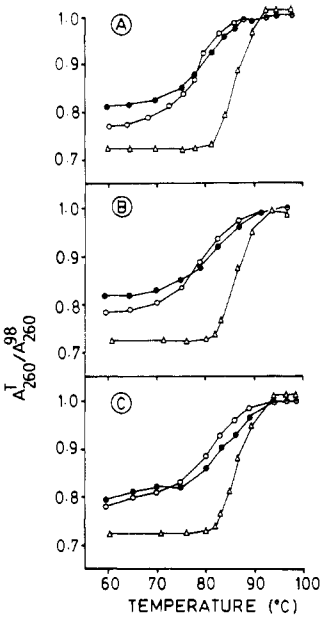

FIGURE 4: Thermal denaturation of reassociated *Panagrellus* DNA. DNA fragments 270 nucleotides (○) and 1284 nucleotides (●) after boiling were reassociated to various C_0t values and bound to hydroxylapatite. Reassociated fragments were eluted at 60 °C with 0.5 M phosphate buffer, dialyzed into 0.12 M phosphate buffer, and thermally denatured. Native DNA (△) was present during each melt. Panels A, B, and C show the thermal denaturation profiles of DNA incubated to a C_0t of 0.5, 10, and 100, respectively.

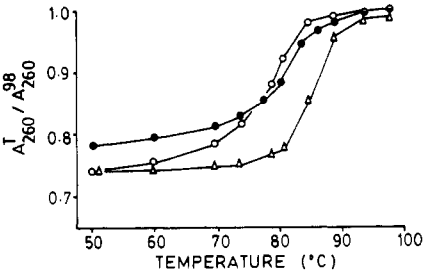

FIGURE 5: Thermal denaturation of reassociated *Panagrellus* DNA. DNA fragments 252 nucleotides (○) and 2304 nucleotides (●) after boiling were stripped of zero-time binding DNA, denatured, incubated to C_0t 0.2, and chromatographed on hydroxylapatite. Reassociated fragments were eluted at 60 °C with 0.5 M phosphate buffer, dialyzed into 0.12 M phosphate buffer, and thermally denatured. Native DNA (△) was present during the melt.

Table II: Thermal Denaturation Parameters of Reassociated *Panagrellus* DNA

C_0t (M s)	fragment size (nucleotides)	T_m (°C)	% mismatch[a]	hyperchromicity	rel hyperchromicity[b]
0.5	270	78.8	5.3	0.230	0.816
	1284	80.4	6.6	0.191	0.675
10	270	79.6	4.5	0.216	0.766
	1284	81.6	4.9	0.182	0.645
100	270	80.0	4.1	0.222	0.797
	1284	83.0	3.5	0.207	0.734
0.2[c]	252	78.5	4.2	0.259	0.918
	2304	80.7	5.8	0.218	0.826

[a] The percent mismatch is computed from the difference between the T_m of native DNA (86.0 °C) and the T_m of reassociated DNA after correcting for fragment length using $\Delta T = 650/L$, where ΔT is the depression in T_m for short fragments and L is the fragment length in nucleotides (Britten et al., 1976). [b] The hyperchromicity of reassociated DNA is compared with the hyperchromicity of native DNA in each melt. [c] The DNA was stripped of foldback sequences prior to reassociation to C_0t 0.2.

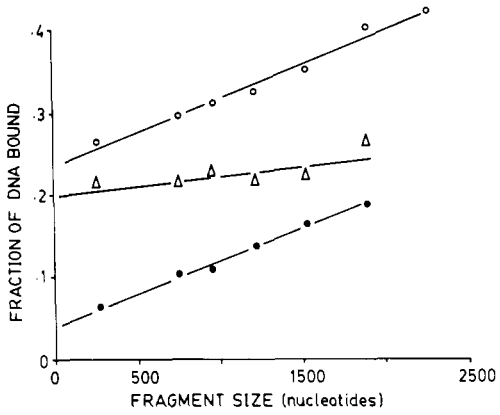

FIGURE 6: The fraction of tracer DNA bound to hydroxylapatite after incubation with excess short driver DNA as a function of the size of tracer DNA. *Panagrellus* [^{125}I]DNA fragments of various lengths were fractionated from an alkaline sucrose gradient and dialyzed against 0.12 M phosphate buffer. For each point (○), 7000 cpm of [^{125}I]DNA of a particular length was mixed with 2 OD/mL of unlabeled DNA (241 nucleotides long), denatured, and incubated to C_0t 0.5. At this C_0t value only zero-time and repetitive DNA will reassociate. Each point is the mean of two determinations. Tracer DNA (7000 cpm) of specified lengths was mixed with 10 μg of *E. coli* DNA, denatured, and applied to hydroxylapatite at 60 °C with the duration at 60 °C being 1 to 2 min (●). The expression $R = (B - Z)/(1 - Z)$, where R = fraction of fragments containing a repetitive element, Z = fraction of zero-time binding DNA, and B = fraction of DNA binding at C_0t 0.5, was used to correct for the presence of zero-time DNA in each of the DNA driven reactions (△).

[^{125}I]DNA that was labeled in vitro was used. Control experiments showed that the kinetic properties of highly sheared [^{125}I]DNA were similar to unlabeled DNA. First, at C_0t 0.5 the extent of renaturation was 0.270 and 0.265 for [^{125}I]DNA and unlabeled DNA, respectively. Second, when [^{125}I]DNA was mixed with unlabeled DNA of the same size, denatured and incubated to C_0t 1385, the extent of the reaction was approximately the same with 91% and 89% of the unlabeled and labeled DNA reannealing.

Tracer fragments of various defined lengths were obtained by fractionating [^{125}I]DNA on a preparative alkaline sucrose gradient. Individual mixtures of labeled DNA and 10000-fold excess of small DNA fragments were denatured, reassociated to a C_0t of 0.5, and assayed by hydroxylapatite chromatography. The relative amount of bound labeled DNA is plotted as a function of fragment length (Figure 6, open circles). These data yield a straight line (r = 0.99) with a slope of 7.97×10^{-5}. The y intercept is 0.238. When the bound fraction is corrected for zero-time binding (Figure 6, closed circles), a straight line (r = 0.73) with a slope of 2.28×10^{-5} is obtained (Figure 6, triangles). There is no clear break in this curve at 1000 nucleotides. Such a change in slope would have been characteristic of DNA like that from *Xenopus* which has short period interspersion.

As noted above, at C_0t 10^{-3}, with fragments of about 290 nucleotides long, 7.55 ± 0.4% of the total *Panagrellus* DNA bound to hydroxylapatite. At this C_0t value some bimolecular reassociation may have occurred. However, when [^{125}I]DNA was passed over hydroxylapatite at C_0t 10^{-6}, 8.5% of the labeled DNA was retained. This close agreement suggests that foldback DNA is the predominant duplexed DNA species at C_0t 10^{-3}. The DNA that binds to hydroxylapatite at C_0t 2×10^{-3} was examined further (Table III). The S_1 nuclease digestion of isolated zero-time binding material yields 3.9% foldback sequences in fragments that are 2300 nucleotides long. A value of 4% for foldback sequences in total DNA is also obtained by extrapolating the zero-time binding values

Table III: Properties of *Panagrellus* Foldback DNA

	initial fragment size	
	252 nucleotides	2304 nucleotides
fraction of DNA bound at C_0t 2×10^{-3}	0.069	0.205
fraction of S_1 nuclease resistant foldback DNA	0.266	0.192
fraction of foldback DNA per genome	0.018	0.039
size of S_1 nuclease resistant foldback DNA (nucleotides)	82	185
no. of nucleotide pairs in foldback DNA per genome	1.48×10^6	3.29×10^6
no. of inverted repeats per genome	1.80×10^4	1.69×10^4
mean nucleotide distance between foldback DNA foci	9.1×10^3	9.45×10^3

in Figure 6 (closed circles) to the y axis. The modal size of the duplexed foldback segments (i.e., hairpin stems) was determined from ultracentrifugal velocity measurements after S_1 nuclease digestion. With large initial fragments of 2300 nucleotides the hairpin stems contain an average 185 nucleotide pairs. Thus, there are 1.69×10^4 inverted repeats per genome in *Panagrellus* (i.e., $0.039 \times 8.22 \times 10^7/185$, where 8.22×10^7 is the genome size in nucleotide pairs). Similar values for both the number of inverted repeats per genome (i.e., $0.018 \times 8.22 \times 10^7/82$) and the extent of spacing between foldback foci were obtained with DNA fragments of 252 nucleotides that had been renatured to C_0t 2×10^{-3} (Table III).

Discussion

In the present study, the complexity of the genome of the free-living nematode *Panagrellus silusiae* has been determined. As measured by hydroxylapatite binding, pressure-sheared *Panagrellus* DNA contains foldback, repetitive, and single copy sequence elements that comprise, respectively, 9.3%, 26.1%, and 61.3% of the genome. The kinetically estimated haploid genome size of *Panagrellus silusiae* (0.097 pg) agrees with the genome size determined by microspectrophotometric techniques (0.09 pg, Pasternak & Haight, 1975). Although $C_0t_{1/2}$ values for unique DNA of other free-living nematodes have not been reported, the DNA reassociation data of single copy DNA of two free-living nematodes, *Panagrellus redivivus* (Searcy & MacInnis, 1970) and *Caenorhabditis elegans* (Sulston & Brenner, 1974), can be used to compute approximate $C_0t_{1/2}$ values of 60 and 90, respectively. Both of these values compare favorably with $C_0t_{1/2}$ 83.3 for the unique DNA of *Panagrellus*.

Brenner (1974) has estimated that the free-living nematode *Caenorhabditis elegans* has about 2000 indispensable genes. Since *Panagrellus* has a similar genome size as *Caenorhabditis* and assuming a mean coding length of 1.2×10^3 nucleotides per structural gene, then about 10% of the single copy DNA in *Panagrellus* would code for a basic repertoire of structural genes. A similar phenomenon in which a small portion of the unique DNA fraction codes for structural genes exists in other eukaryotes (Bishop et al., 1974). For example, in *Drosophila*, about 9% of the single copy DNA could code for 5000 structural genes. Interestingly, yeast, a unicellular eukaryote, has 3000–4000 genes that are probably associated with routine cellular functions (Hereford & Rosbash, 1977). Thus, on the one hand, the number of coding DNA sequences does not readily set simple metazoans apart from a primitive eukaryote such as yeast. On the other, it is probably significant that in yeast its 3000–4000 structural genes represent 40% of the genome, whereas in both the fruitfly and nematode the basic set of structural genes comprises about 10% of the unique DNA.

In general, the characteristics of foldback DNA in *Panagrellus* agree with those found in other organisms. The stems of foldback DNA (inverted repeats) comprise about 4% of the total genome in *Panagrellus*, which approximates the amount that has been observed with various eukaryotes (Wilson & Thomas, 1974). However, the modal stem lengths of inverted repeats vary in different organisms. In *Panagrellus*, after S_1 nuclease digestion of fragments containing 2300 nucleotides that were reannealed to a C_0t 2×10^{-3}, the mean stem length is 185 nucleotide pairs. Values of 150–200 nucleotide pairs per stem have been observed in human and cotton DNA (Dott et al., 1976; Walbot & Dure, 1976), whereas the stems in *Drosophila* and mouse foldback DNA are greater than 1000 nucleotide pairs (Schmid et al., 1975; Cech & Hearst, 1975). The number of inverted repeats per *Panagrellus* genome is about 1.8×10^4 and differs from the amounts that have been found in *Drosophila* ($2–4 \times 10^3$; Schmid et al., 1975), human DNA (2×10^6; Dott et al., 1976), mouse (4×10^4; Cech & Hearst, 1975), and *Xenopus* (10^5; Perlman et al., 1976). Although the function of foldback DNA is unknown, such palindromic stretches are a common component of eukaryotic genomes.

By several criteria, we have demonstrated that the genome of *Panagrellus* lacks extensive short period interspersion of repetitive and nonrepetitive DNA sequence elements. The evidence of this conclusion was based on (1) C_0t plots on hydroxylapatite with DNA fragments of different lengths; (2) the extent of hyperchromicity of DNA that was reassociated to various C_0t values; and (3) the pattern of hybridization of tracer DNA of different sizes after incubation with excess DNA to a low C_0t value.

Data on the hyperchromicity of reassociated *Panagrellus* DNA support the notion that there is little interspersion in the genome of this organism. First, the relative fraction of duplex DNA is not dramatically different between small and large fragments of reassociated repetitive DNA. Second, the relative fraction of duplex DNA does not increase greatly at higher C_0t values. The mean length of duplex DNA in C_0t 0.2 DNA is 1900 nucleotides, i.e., 2300 nucleotides $\times$ 0.826, where 2300 is the nucleotide length of the DNA after boiling and 0.826 is the relative hyperchromicity. Thus, allowing for unreassociated "tails", repetitive DNA of fragments which are at least 2000 nucleotides long contain little if any unique DNA.

Manning et al. (1975) proposed a method of approximating the length of repeated sequences from reassociation data for DNA fragments of different lengths. Their approach assumes that all repeated sequences are of uniform length. By this consideration, the ratio of unique DNA to repeated DNA in reassociated long fragments should be L/R, where L is the DNA fragment length and R is the length of the repeated DNA in the genome. In *Panagrellus*, repeated sequences form about 20% of the genome (Figure 6; y intercept of curve with open triangles). This component increases to 24% of the genome when DNA fragment size is increased to 2000 nucleotides. Therefore we estimate that the length of the repeated elements may be about 10 000 nucleotides long. Although the estimates of the total amount of repeated DNA that were determined from C_0t plots of unlabeled DNA are about 5% longer than the corresponding value for labeled DNA, the relatively small increase in the repeated component with increased fragment size supports the conclusion that in *Panagrellus* the repeated elements are clustered in very long fragments.

At present, it is not possible to define experimentally the pattern of long period interspersion in *P. silusiae* because we have not yet been able to extract in quantity large fragment sizes (i.e., >5000 nucleotides). Notwithstanding, the genome of *P. silusiae* is organized in a pattern that is akin to the type that has been described for *Drosophila*, *Apis*, *Chironomus*, *Achlya*, chicken, and Syrian hamster (Crain et al., 1976a,b; Wells et al., 1976; Hudspeth et al., 1977; Arthur & Straus, 1978; Moyzis et al., 1977). This particular pattern, which may contain a small, but not readily detected, amount of DNA that has short period interspersion, contrasts with the DNA sequence arrangement of many other organisms for which the *Xenopus* genome has been used as the exemplar. If the members of the group that exhibit long period interspersion are exceptions to a more-or-less commonplace pattern and if the long period interspersion organization of DNA is due to selective loss of interspersed single copy spacer regions (Davidson et al., 1977), then these diverse nonconforming organisms provide an interesting example of evolutionary convergence.

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Modulation of Erythrocyte Membrane Proteins by Membrane Cholesterol and Lipid Fluidity†

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ABSTRACT: Human erythrocyte membranes were enriched or depleted of cholesterol and effects on membrane proteins assessed with a membrane-impermeant sulfhydryl reagent, [^{35}S]glutathione–maleimide. Reaction of the probe with intact cells quantifies exofacial sulfhydryl groups and reaction with leaky ghost membranes permits quantification of endofacial sulfhydryl groups. The mean endofacial sulfhydryl titer of cholesterol-enriched membranes exceeded that of cholesterol-depleted membranes by approximately 45 nmol/mg of protein, or 64%. The corresponding exofacial titer of cholesterol-enriched cells was less than that of cholesterol-depleted cells by approximately 0.4 nmol/mg of protein, or 14%. Labeled membranes were examined by autoradiography of sodium dodecyl sulfate–polyacrylamide gel electropherograms to determine the labeling patterns of individual protein bands. Cholesterol enrichment enhanced the surface labeling of Coomassie brilliant blue stained bands 1, 2, 3, and 5, decreased the labeling of band 6, and did not change significantly that of band 4. The results demonstrate that changes in membrane cholesterol which influence lipid fluidity can alter the surface labeling of both intrinsic and extrinsic membrane proteins.

The fluidity of the lipids of biological membranes influences membrane proteins in several ways. Considerable evidence that the lateral and rotational movements of the proteins are affected has been reviewed (Edidin, 1974; Cherry, 1976). More recently, the results of fluorescence studies (Borochov & Shinitzky, 1976; Shinitzky & Rivnay, 1977) indicate that alteration of lipid fluidity also modulates the availability of protein substituents at the membrane surfaces. Thus, enrichment of human erythrocyte membranes in cholesterol, which decreases bulk lipid fluidity, enhanced the surface exposure of membrane proteins, and conversely, depletion in cholesterol decreased the surface exposure. A working hypothesis to explain these findings (Borochov & Shinitzky, 1976; Shinitzky & Rivnay, 1977) assumes that membrane proteins, as amphipathic molecules, are influenced strongly by their relative interactions with bulk membrane lipids and ambient water. Hence decreased lipid fluidity, which corresponds to enhanced lipid–lipid interactions, may result in decreased protein–lipid interactions and increased protein–water interactions. The proteins, or portions thereof, may thus be displaced toward the membrane surfaces.

To test the working hypothesis further and to quantify more precisely the effects of lipid fluidity on the surface availability of protein substituents, we have applied a membrane-impermeant sulfhydryl reagent, [^{35}S]glutathione–maleimide ([^{35}S]GSmal).[1] Sulfhydryl groups reactive at the exofacial (outer) and endofacial (inner) surfaces of human erythrocyte membranes are quantified by labeling separately intact cells and leaky ghost membranes, and mean values of 1.6 and 28 amol/cell, respectively, have been reported for the outer and inner surfaces (Abbott & Schachter, 1976). In the experiments described below, human erythrocytes were first treated to alter the membrane cholesterol to phospholipid molar ratio (C/PL) and the effects on the surface –SH titers and on the –SH groups of individual protein bands separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis were determined.

Materials and Methods

Sulfhydryl Reagent. The membrane-impermeant –SH reagent [^{35}S]glutathione–maleimide I was prepared and characterized as previously described (Abbott & Schachter, 1976).

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[1] Abbreviations used: C/PL, cholesterol to phospholipid molar ratio; GSmal, glutathione–maleimide; PBS, phosphate-buffered saline; $NaDodSO_4$, sodium dodecyl sulfate.

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