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DNA sequence organization in the water mold *Achlya*

(reassociation kinetics/interspersion/fungi)

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ABSTRACT **Experiments are described that characterize the organization of DNA sequences in the water mold *Achlya bisexualis*. These experiments demonstrate that repetitive and single copy sequences in the *Achlya* genome are arranged in a long-period interspersion pattern. Estimates of the spacing intervals between repetitive and single copy DNA indicate, however, that the interspersion pattern in *Achlya* is longer than has been previously reported in other eukaryotes. These data and measurements of structural gene expression in *Achlya* [Timberlake, W. E., Shumard, D. S. & Goldberg, R. B. (1977) *Cell* 10, 623–632] make it difficult to propose a regulatory function for repeated DNA in this eukaryote.**

Two general patterns of DNA sequence arrangement have been found in eukaryotic chromosomes (1). Genomes in which a major fraction of the DNA consists of repetitive sequences [averaging 200–400 nucleotide pairs (Nt pr)] linked to single copy DNA at intervals of less than 2000 Nt pr are said to have the short-period interspersion pattern (2). Short interspersion is found in representative species of all major animal phyla and flowering plants (1, 3). Genomes that lack short repetitive sequences but contain long stretches (>4000 Nt pr) of relatively nondivergent repeated DNA, contiguous to single copy sequences at long intervals (>10,000 Nt pr), are said to have the long-period interspersion pattern (4, 5). To date, only a few insect species—e.g., *Drosophila*—with small genome sizes (<0.4 pg) have been found to have this interspersion pattern (4–7). What relationship, if any, these very different patterns of DNA sequence arrangement have to transcriptional processes and gene regulation in eukaryotes is not presently understood.

True fungi represent the only major group of eukaryotes for which little is known about the organization of repetitive and single copy DNA. These syncytial microorganisms have very small genomes (<0.05 pg) and generally low quantities (5–20%) of repetitive DNA (8). The water mold *Achlya* is an Oomycete in which sexual reproduction is regulated by well-characterized steroid hormones (9). This fungus is biologically simple (only nine cell types), easily studied by biochemical and genetic methods, and readily induced to undergo specific developmental programs (10). *Achlya*, therefore, represents a novel model system with which to investigate steroid-mediated gene regulation. Recently, we determined that mRNA transcription and maturation in *Achlya* is much less complex than that found in animal cells (11). Here we report experiments that characterize the pattern of DNA sequence organization in the *Achlya* genome. The results reveal that repetitive and single copy sequences are interspersed in *Achlya*, but at longer intervals than have been previously observed in plant and animal genomes.

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MATERIALS AND METHODS

Growth of *Achlya* Cultures and DNA Labeling. *Achlya bisexualis* strain 65-1 (♀) was grown as described (10, 11). DNA was labeled by incubating cultures with [^{3}H]thymidine (New England Nuclear, 40 mCi/mmol) at 20μCi/ml or carrier-free $Na^{32}PO_4$ (New England Nuclear) for 24 hr at 30°. Mycelia were harvested, washed, and stored at −70°.

Purification of Nuclear DNA. Cellular DNA was extracted from mycelia by a modified 8 M urea/0.24 M phosphate buffer (PB) procedure of Britten *et al.* (12). The details of this procedure have been described (13). Nuclear DNA was separated from mitochondrial DNA by three cycles of preparative CsCl density centrifugation (14). The physical parameters of the purified nuclear DNA were: (*i*) buoyant density in CsCl, 1.703 g/ml; (*ii*) temperature at which 50% of the DNA denatures (t_m) in 0.12 M PB, 88.5°; (*iii*) hyperchromicity as percentage of final absorbance, 28%; and (*iv*) modal single-strand fragment length [(11,000 nucleotides (Nt)].

Shearing of DNA. DNA was sheared to different single-strand sizes in the Virtis 60 homogenizer (15, 16). The modal single-strand fragment lengths were ascertained by analytical alkaline band sedimentation velocity in the model E ultracentrifuge (17) or by alkaline sucrose density centrifugation (2).

Reassociation Analysis. Detailed descriptions of the methods used in our laboratory for conducting and analyzing DNA·DNA reassociation experiments have recently been published (3, 11, 13). Typically, samples of DNA were denatured and incubated in 0.12 M PB at 60° or 0.41 M PB at 68° to the desired C_0t [initial DNA concentration (mol of nucleotide/liter) × time (sec)] value. All reactions in 0.41 M PB were converted to equivalent C_0t values by multiplying by a factor of 5 (15). Hydroxyapatite (HAP) chromatography was used to separate duplex-containing DNA fragments from DNA fragments that were entirely single-stranded (15). All DNA fragments exceeding 1000 Nt in length were eluted from HAP without the aid of air pressure in order to minimize shearing (3). Recovery of DNA from HAP (either A_{260} or cpm) was always 95–105% of the input mass.

RESULTS

Reassociation Kinetics of Short DNA Fragments. Fig. 1*A* portrays the reassociation kinetics of 200-Nt DNA fragments. The least-squares analysis indicates the presence of two second-order components, designated repetitive and single copy.

Abbreviations: Nt pr, nucleotide pairs; PB, sodium phosphate buffer; t_m, temperature at which 50% of the DNA denatures; Nt, nucleotides; C_0t, initial DNA concentration (mol of nucleotide/liter) × time (sec); HAP, hydroxyapatite.

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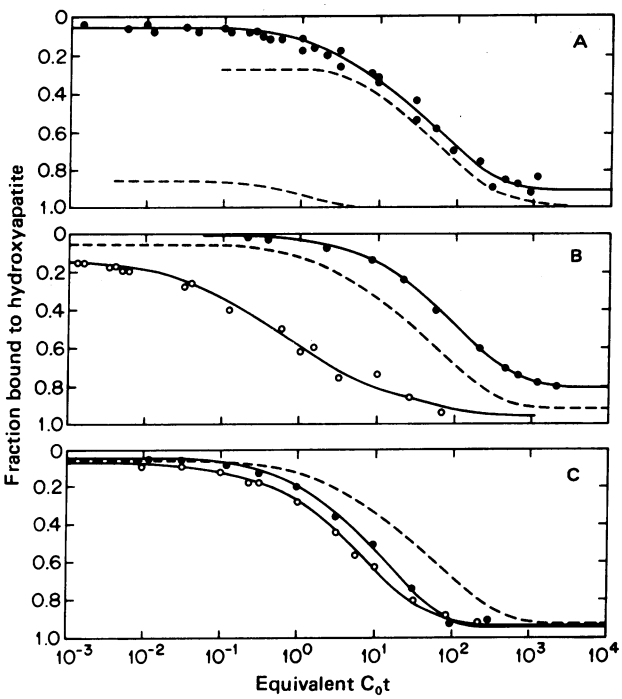

FIG. 1. Reassociation kinetics of *Achlya* nuclear DNA. (*A*) Reassociation of 200-Nt total DNA fragments (●). The solid curve portrays the best least-squares solution of the renaturation data yielding two second-order components represented by the dashed curves: 14% with a K of 1.48 M^{-1} sec^{-1} (repetitive) and 73% with a K of 0.022 M^{-1} sec^{-1} (single copy). Approximately 5% of the DNA bound to HAP at the earliest C_0t measured (10^{-3}) but 8% failed to bind at a C_0t of 10^3. (*B*) Reassociation of isolated kinetic components of nuclear DNA. Renaturation of isolated 200-Nt ^{3}H-labeled repetitive DNA sequences in the presence of a 135-fold excess of unlabeled total 200-Nt DNA fragments (○). The repetitive DNA sequences represented a fraction of 200-Nt [^{3}H]DNA bound to HAP at a C_0t of 1 (12.3%). The solid curve represents the best least-squares solution of the data yielding three second-order components: 17% with a K of 39.7 M^{-1} sec^{-1} (repetitive-1), 48% with a K of 1.39 M^{-1} sec^{-1} (repetitive-2), and 16% with a K of 0.022 M^{-1} sec^{-1} (residual single copy). An additional 16% of the repetitive tracer bound to HAP at a C_0t of 10^{-3} (foldback). Renaturation of isolated 135-Nt single copy [^{3}H]DNA sequences in the presence of a 700-fold excess of total 200-Nt DNA fragments (●). The single copy sequences represented a fraction of 200-Nt [^{3}H]DNA not bound to HAP at a C_0t of 10 (67.9%). The solid curve represents the best least-squares solution for the two second-order components: 8% with a K of 1.73 M^{-1} sec^{-1} (residual repetitive) and 74% with a K of 0.012 M^{-1} sec^{-1} (single copy). The dashed curve portrays the renaturation of the driver DNA. (*C*) Reassociation of long DNA fragments. The solid curves portray the best two-component least-squares solution to the renaturation data. The dashed curve represents the reassociation of 200-Nt DNA for comparison. The best least-squares solution to the 2500-Nt renaturation data (●) is: 10% with a K of 5.8 M^{-1} sec^{-1} (repetitive), 81% with a K of 0.09 M^{-1} sec^{-1} (single copy), and 5% early binding. The best solution to the 7500-Nt renaturation data (○) is: 12% with a K of 4.54 M^{-1} sec^{-1} (repetitive), 73% with a K of 0.143 M^{-1} sec^{-1} (single copy), and 9% early binding. No difference in the binding of long DNA fragments to HAP resulted by lowering the annealing and fractionation criterion to 50°. This was expected because *Achlya* repetitive DNA sequences have undergone minimal divergence (see Table 1). Using the formula $\Delta t_m = (650/N)$ (15), we estimated that repetitive duplexes of a minimum of 17 Nt pr in length will be bound to HAP at the 50° criterion.

The single copy component comprises the majority of annealing DNA sequences, accounting for about 75% of the binding to HAP.

A more sensitive experiment describing the reassociation kinetics of *Achlya* DNA is presented in Fig. 1*B*. Here, enriched ^{3}H-labeled repetitive and ^{3}H-labeled single copy DNA fractions, previously prepared from total [^{3}H]DNA by annealing and HAP chromatography, were reassociated in the presence of an excess of unlabeled nuclear DNA. Allowing for a slight contamination with repetitive DNA sequences resulting from the HAP fractionation procedure, the ^{3}H-labeled single copy fraction reassociates as a single kinetic component with a K of 0.018 M^{-1} sec^{-1} (corrected to 200-Nt fragment length). Relative to the reassociation of *Escherichia coli* DNA, we compute the genome size of *Achlya* to be 4.19×10^7 Nt pr or 0.046 pg, a value similar to that of other representatives of the fungal kingdom (8). The ^{3}H-labeled repetitive fraction, on the other hand, binds to HAP with heterogeneous reassociation kinetics. This fraction contains sequences that bind instantaneously to HAP (foldback) as well as two second-order components. These repetitive components, referred to as repetitive-1 and repetitive-2, have average reiteration frequencies of 2200 and 80, respectively.

Table 1 summarizes the experiments shown in Fig. 1 *A* and *B*. These data show that *Achlya* has a very simple genome with a small quantity of repetitive DNA but has general features analogous to more complex plant and animal genomes—i.e., simple sequence, middle repetitive, single copy, and foldback DNA.

Reassociation Kinetics of Long DNA Fragments. The extent to which repetitive and single copy sequences are interspersed can be ascertained from a comparison of the reassociation kinetics of short (<300 Nt) and long (>1500 Nt) DNA fragments (2). The self-reassociation kinetics of 2500 Nt and 7500 Nt DNA fragments are presented in Fig. 1*C*. The least-squares analysis indicates the presence of two second-order components (repetitive and single copy) in quantities similar to those obtained from the reassociation kinetics of 200-Nt DNA fragments (Fig. 1*A*). Although there is an increase in the rate at which *Achlya* DNA binds to HAP at longer fragment lengths, the increase can be accounted for by the effect of fragment length on reassociation rate (18). If we impose upon the data of Fig. 1*C* a least-squares solution using theoretical repetitive and single copy rate constants computed from the 200-Nt data presented in Table 1 ($K_L = [L/200]^{1/2} \times K_{200}$), there is no significant increase in root-mean-square error. These results demonstrate that populations of *Achlya* DNA molecules (7500 Nt or less) contain fragments that are almost exclusively repetitive or single copy with minimal linkage between the two.

A more direct assay of the degree to which repetitive and single copy sequences are interspersed is presented in Fig. 2. In this experiment, [^{3}H]DNA fragments were annealed with an excess of repetitive driver DNA (4). Binding of [^{3}H]DNA to HAP, therefore, is solely the result of the reassociation of repetitive sequences. Hence, any increase in HAP binding of a long DNA tracer in comparison to a shorter one must be a consequence of the linkage of repetitive and single copy sequences on the same DNA molecule (2). We observe no significant difference in the HAP binding of [^{3}H]DNA populations over a 25-fold range of fragment sizes (200–5000 Nt). These results and those of the self-reassociation experiments demonstrate that we are unable to detect any significant interspersion of repetitive and single copy DNA in *Achlya* within the domain of fragment lengths used—i.e., up to 7500 Nt.

Size of Reassociated Repetitive Duplexes. A direct demonstration that repetitive sequences are contained within the full length of *Achlya* reassociated DNA molecules is presented in Fig. 3. In this experiment, we annealed 7500-Nt DNA fragments to a C_0t value at which only repetitive sequences react and then treated them with S1 nuclease. Our S1 nuclease

Table 1. Sequence components of the *Achlya* genome

Component	Fraction of genome*	K†	$C_0t_{1/2}$†	Average no. copies‡	Complexity§
Foldback	0.02				
Repetitive-1¶	0.02	39.7	0.025	2200	380
Repetitive-2¶	0.14	1.39	0.72	80	7.6×10^4
Single copy	0.82	0.018	55.6	1	3.4×10^7

* Computed from the reassociation analysis of 200-Nt total DNA fragments, the renaturation kinetics of the ^{3}H-labeled repetitive and single copy fractions, and the yield of repetitive and single copy DNA by reassociation and HAP fractionation. Estimates represent genomic values because repetitive and single copy sequences are not interspersed at short DNA intervals (<2000 Nt) in the *Achlya* genome.

† Second-order rate constant (M^{-1} sec^{-1}) for 200-Nt fragments in whole DNA at a 60°, 0.18 M Na^+ criterion. The $C_0t_{1/2}$ is the reciprocal of the K value.

‡ Computed from the ratio of repetitive and single-copy rate constants in whole DNA (3, 13).

§ Values represent genomic complexity in Nt pr. Calculated from the genome size of 4.19×10^7 Nt pr, the genomic fraction of each component, and the average number of copies per genome (3).

¶ The t_m of reassociated repetitive DNA is 3° lower than that of native DNA of the same fragment length. Thus, families of *Achlya* repetitive DNA have undergone minimal nucleotide sequence divergence.

conditions remove >98% of the single-stranded DNA but leave repetitive duplexes intact (19). The S1 nuclease-resistant duplexes were concentrated on HAP and their size distribution was determined by agarose gel filtration (3, 16). Over 85% of the repetitive duplexes were excluded from the agarose column. These duplexes were collected and their modal single-strand fragment length was measured to be 7500 Nt, a value equal to the original size of the DNA population. The sensitivity of this measurement is such that differences between DNA populations ranging from 6000 Nt (17 S) to 10,000 Nt (21 S), a 20% difference in S value, would be difficult to detect by our sizing methods. However, this result demonstrates that most of the S1 nuclease-resistant repetitive duplexes are long and within the size range of the original DNA population. It is unlikely that the small fraction of duplexes that are included in the column

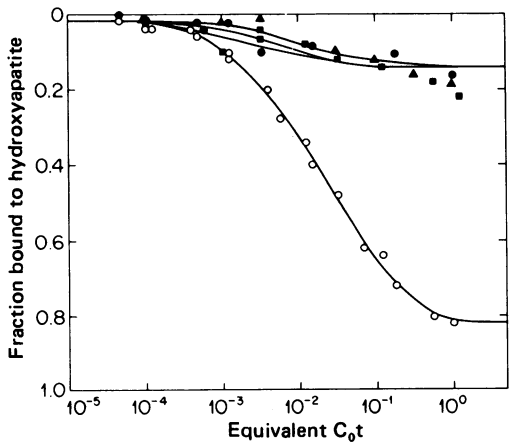

FIG. 2. Reassociation kinetics of total nuclear [^{3}H]DNA fragments of various lengths with 200-Nt ^{32}P-labeled repetitive DNA. Tracer DNA populations 200-Nt (●), 1500-Nt (■), and 5000-Nt (▲) in length were prepared by shearing total [^{3}H]DNA. Foldback sequences were removed from each [^{3}H]DNA population by HAP fractionation at C_0t of $\leq 10^{-5}$ and the unbound portion was reassociated in the presence of a 300-fold excess of 200-Nt ^{32}P-labeled repetitive driver DNA. The driver DNA represented a fraction of [^{32}P]DNA bound to HAP at a C_0t of 1 and stripped of foldback at a C_0t of 10^{-5}. The renaturation of the ^{32}P-labeled repetitive driver DNA is portrayed by a two-component least-squares solution (○): 32% with a K of 265 M^{-1} sec^{-1} (repetitive-1) and 50% with a K of 19.1 M^{-1} sec^{-1} (repetitive-2). The renaturation of each [^{3}H]DNA population is portrayed by a two-component least-squares solution to the data points. The observed percentage of each [^{3}H]DNA tracer bound to HAP at a C_0t of 1 was 18, 22, and 15% for 200-Nt, 1500-Nt, and 5000-Nt [^{3}H]DNA tracers, respectively.

(maximally 15%) and display a continuum of sizes is a major feature of the *Achlya* genome but probably represent short foldback sequences and duplexes resulting from the size distribution of the original randomly sheared 7500-Nt DNA population. This experiment therefore demonstrates that, on the average, repetitive sequences in *Achlya* extend for at least 6,000–10,000 Nt pr without interruption by single copy DNA and that short interspersed repetitive sequences—i.e., 300 Nt pr—are not represented in the *Achlya* genome.

A Test for Single Copy Sequences Contiguous to Repetitive DNA. Although the vast majority of single copy sequences are not adjacent to repetitive DNA at fragment lengths up to 7500 Nt, these sequences were undoubtedly interspersed in the genome (4). Interspersion at long-sequence intervals can be directly tested for by significantly enriching a randomly sheared population of long DNA molecules for repetitive sequences. Then, after shearing, the amounts of repetitive and single copy DNA can be assayed for kinetically (5). This establishes an upper limit of the extent to which single copy sequences are contiguous to repetitive DNA at the fragment length used.

Fig. 4 presents the results of such an enrichment experiment with *Achlya* DNA. We kinetically determined the DNA sequence representation in a population of 5000-Nt [^{3}H]DNA fragments enriched 7-fold for repetitive sequences plus any linked single copy DNA. The least-squares analysis clearly demonstrates that the majority of DNA fragments binding to HAP do so as a result of the reassociation of repetitive DNA sequences. However, a small fraction of DNA fragments (maximally 21%) become HAP-bound with kinetics consistent with those of single copy sequences. We did not previously detect these contiguous single copy sequences because they represent only 3% (0.21 × 0.142 × 100) of the original 5000-Nt [^{3}H]DNA population. This is within the margin of error of reassociation experiments analogous to those presented in Figs. 1*C* and 2. Single copy and repetitive sequences are therefore interspersed in the *Achlya* genome, but as an upper limit only 3.6% (0.03/0.82 × 100) of the single copy DNA is continguous to repetitive sequences at a 5000-Nt fragment length. Clearly, the genomic lengths of single copy and repetitive DNA in *Achlya* must be very long indeed.

DISCUSSION

The *Achyla* genome is simple in comparison to those of more advanced eukaryotic cells. Nuclei of this fungus contain only 4.19×10^7 Nt pr of DNA, 82% of which is single copy. Previous measurements have demonstrated that most mRNA molecules in *Achlya* (average size, 1100 N) are transcribed from single

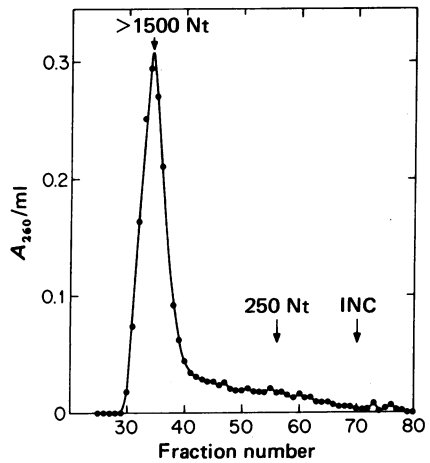

FIG. 3. Size distribution of S1 nuclease-resistant repetitive duplexes. DNA fragments (7500 Nt) were reassociated to a C_0t of 0.3 (a C_0t at which over 75% of *Achlya* repetitive sequences form duplex structures but only 3% of single copy sequences can react) at 60° in 0.18 M NaCl/6 mM 1,4-piperazinediethanesulfonic acid (Pipes), pH 6.7, directly treated with S1 nuclease according to the mild conditions of Britten *et al.* (19), and collected by HAP chromatography (13, 16). Approximately 15% of the DNA was resistant to S1 nuclease. The S1 nuclease-resistant repetitive duplexes were then chromatographed over an Agarose A-50 column (3, 16). The arrows represent elution positions of calf thymus DNA markers. The $C_0t_{1/2}$ of 7500-Nt DNA fragments containing only sequences of the repetitive-2 component is 0.12. Because a C_0t of 0.3 is almost 3 times the $C_0t_{1/2}$ of the major repetitive component, first collision overlap should be minimal and long hydropolymers of repetitive DNA can form as a result of secondary and tertiary collisions (20). In the absence of S1 nuclease, 18% of 7500-Nt DNA fragments bind to HAP at a C_0t of 0.3 (Fig. 1*C*). By extrapolation from the S1 nuclease data, we estimate that more than 80% [(0.15/0.18) × 100] of the C_0t 0.3 non-S1 nuclease-digested DNA fragments are in duplex structures, suggesting an average duplex length in excess of 6000 Nt pr (7500 Nt × 0.80). The 20% S1 nuclease-sensitive regions are most likely due to overlap and the presence of linked single copy DNA (see Fig. 4). The fact that the average length of C_0t-0.3 repetitive duplexes exceeds 6000 Nt pr was confirmed by measuring the single-strand fragment length of S1 nuclease-resistant duplexes excluded from the agarose column (see *text*). After normalization of fragment lengths, the t_m (86°) of repetitive duplexes excluded from agarose (fraction 34) was similar to the t_m of repetitive duplexes included in the column (fractions 46–50 and 56–60). In contrast to plant and animal repetitive DNA sequences (3, 16, 19), therefore, reassociated repetitive duplexes of *Achlya* DNA contain the same degree of mismatching (3%) regardless of length. Inc, inclusion fraction.

copy DNA (11). Hence, there are potentially 30,000 distinct structural genes in the entire genome. We have recently determined that vegetatively growing *Achlya* cells express approximately 2000 single copy sequences, which is only 7% of the entire genomic complexity (11). While we do not know the total number of genes that are expressed during the complete developmental cycle, this value will probably be significantly less than 30,000.

Two distinct repetitive sequence classes are found in *Achlya*, but the total fraction of repeated DNA (16%, excluding foldback) and repetitive sequence complexity (8×10^4 Nt pr) are low. Repeated DNA is transcriptionally active in *Achlya*, coding for both mRNA and rRNA (11, 21). Exactly what proportion of the repeated DNA represents multigene coding sequences and associated spacers is unknown. However, from what is known about just one multigene family (ribosomal DNA) in *Dictyostelium*, an organism with a rRNA processing mechanism, genome size, and genomic complexity similar to those of *Achlya*, this value could exceed 50% of the repeated DNA (22–24).

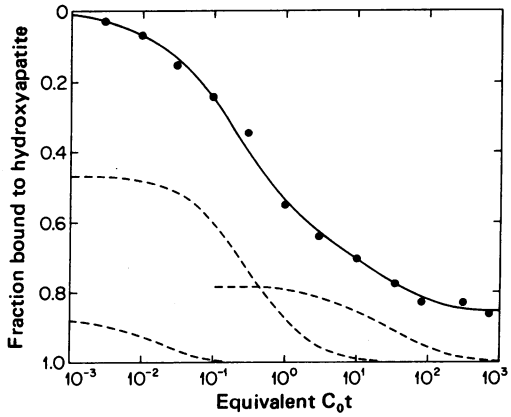

FIG. 4. Reassociation kinetics of DNA sequences contained within an enriched repetitive fraction. [^{3}H]DNA fragments (5000 Nt) stripped of foldback sequences were renatured to a C_0t of 0.3 in the presence of a 60-fold excess of purified ^{32}P-labeled repetitive driver DNA (see Fig. 2). [^{3}H]DNA fragments bound to HAP (14.2%) were sheared to 475 Nt and annealed in the presence of a 2000-fold excess of unlabeled 230-Nt total nuclear DNA. The solid curve represents a three-component least-squares solution to the data points (●) with the rate of each component fixed at the value obtained from the reassociation analysis of isolated kinetic fractions (see Fig. 1*B*). These rate constants were adjusted for the effects of fragment length on renaturation according to the relationship: $K_{475} = K_{200}\,[(230/200)^{1/2}(475/230)]$. The dashed curves represent elements of the overall solution: 13% with a K of 89.8 M^{-} sec^{-1} (repetitive-1), 53% with a K of 3.14 M^{-1} sec^{-1} (repetitive-2), and 21% with a K of 0.043 M^{-1} sec^{-1} (single copy).

Repetitive and single copy DNA in *Achlya* are clearly not organized in a short-period interspersion pattern. We find no evidence for a discrete class of 200- to 400-Nt pr repeated DNA or significant linkage of repetitive and single copy sequences on DNA fragments 7500 Nt in length or less. Nor do we observe any short-period interspersion when reassociation conditions are adjusted so that very short (17–20 Nt pr) and/or divergent (30–40% mismatching) repetitive duplexes can be detected (see legend to Fig. 1*C*). *Achlya* therefore represents the first non-insect eukaryote that has repetitive and single copy sequences arranged in a long interspersion pattern. Consistent with this pattern, repetitive sequences in *Achlya* extend for at least 6000 Nt pr, are nondivergent, and are contiguous to single copy DNA at long intervals. We find this result surprising because the *Dictyostelium* genome is organized in a short-period interspersion pattern (24).

It is not possible at present to directly determine the genomic lengths of repetitive and single copy sequences in *Achlya*, but quantitative aspects of our data can be used to arrive at an approximation. The experiment presented in Fig. 4 demonstrated that only 3.6% of the single copy sequences in *Achlya* (3% of the genome) are linked to repetitive DNA at a 5000-Nt fragment length. This value (Y), which is our best estimate, is a function of the total number of interspersed sequences (repetitive or single copy) in the *Achlya* genome (N), the genome size (G), and the DNA fragment length (L) used for the determination (5). Assuming random interspersion, we can use the relationship $Y = LN/G$ derived by Crain *et al.* (5) to calculate that there are approximately 250 interspersed sequences in the *Achlya* genome. We believe that this computation is valid because the theoretical relationship used has been verified experimentally in *Drosophila* DNA (4, 5). Using 250 interspersed sequences as our best approximation, we calculate that the average genomic lengths of repetitive and single copy DNA in *Achlya* are 2.7×10^4 and 1.35×10^5 Nt pr, respectively. These values are about 5 times longer than has been estimated for

repetitive and single copy DNA in *Drosophila* by using the same approach (4, 5). Although these size approximations cannot as yet be experimentally confirmed, they serve to indicate that, in *Achlya*, repetitive and single copy DNA sequences are very long, much longer than have been estimated for other eukaryotes with the long-period interspersion pattern.

Regardless of the exact lengths of repetitive and single copy DNA or those of individual sequences, it is difficult to reconcile the pattern of sequence organization in *Achlya* with a regulatory function for repeated DNA (25). The numbers of repetitive sequences contiguous to single copy DNA is an order of magnitude lower than the number of structural genes known to be expressed at just one developmental stage. Size estimates of mRNA and nuclear RNA fail to reveal any polycistronic mRNA or giant primary nuclear transcripts (11). RNA sequences found in the nucleus are virtually identical to those being translated on polysomes(11) and the complexity and amounts of repeated DNA suggest a narrow range of functions. While it is possible that there are short (<15 Nt pr) interspersed repetitive sequences or a rapid processing mechanism involving very large nuclear RNAs, we are left with a multicellular eukaryote whose DNA sequence organization and transcriptional processes provide no clues as to the molecular basis of gene regulation. How *Achlya* regulates its genomic activity and the relationship between gene regulation in *Achlya* and those of other organisms, both prokaryotic and higher eukaryotic, remain to be learned.

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