# Low Repetitive DNA Content in *Aspergillus nidulans*

Abstract. *DNA-DNA reassociation experiments show that the genome of* Aspergillus nidulans *consists of approximately 97 to 98 percent unique and 2 to 3 percent reiterated sequences. The reiterated DNA sequences have a complexity of about 11,000 base pairs and are repeated approximately 60 times per haploid genome. Ribosomal RNA-DNA hybridization experiments indicate that most of the repetitive DNA codes for ribosomal RNA.*

The genomes of all of the eukaryotic organisms examined to date are comprised of both unique and reiterated DNA sequence components (*1-5*). The unique DNA contains the majority of the sequences coding for messenger RNA (mRNA) (*2, 6-9*) while the reiterated component contains some structural genes, such as those coding for histones (*10*), as well as the genes for ribosomal and transfer RNA (rRNA and tRNA) (*11*). However, a majority of the repetitive DNA sequences do not code for mRNA, rRNA, or tRNA. In many eukaryotes, a large fraction of the reiterated DNA consists of short, evolutionarily diverged sequences interspersed among single-copy DNA sequences at short intervals (*3, 12-14*). That this pattern of organization of repetitive and unique DNA sequence components has been observed in representatives of the protista, plantae, and animalia constitutes support for its having a high degree of selective value and implies an important cellular function. Britten and Davidson (*13, 15*) have proposed that some of the moderately repeated sequences may have a central role in coordinate gene control. Their hypothesis is especially attractive in that it explicitly provides mechanisms by which unlinked structural genes could be identified by the cell, through their linkage to repetitive sequence elements, as members of a coordinately acting gene set.

Only a small amount of information exists concerning the nature or organization of repetitive DNA sequence components in the fungi, even though several of these organisms, such as *Neurospora*, *Saccharomyces*, and *Aspergillus*, have been exceedingly useful in the genetic analysis of genome organization and gene control (*16*). Reassociation analysis of DNA from several fungi including *Achlya* (*5*), *Phycomyces* (*4*), *Saccharomyces* (*7, 17*), and *Neurospora* (*18*) has shown that these organisms typically have small genomes (< 0.05 pg) and contain limited amounts (5 to 20 percent) of repetitive DNA. The DNA sequence organization of only one of these, the water mold *Achlya*, has been investigated in detail (*5*). While 16 percent of the *Achlya* DNA consists of repetitive sequences, these are not organized in the short period interspersion pattern characteristic of higher plants and animals (*5*).

The fungi have been observed to share many properties with more advanced eukaryotes in terms of their cellular and genetic organization. They contain chromosomes, undergo mitosis and meiosis, and possess unlinked gene sets that are coordinately regulated (*16*). In examining the genome of one of these organisms, *Aspergillus nidulans*, for the presence of repetitive DNA, I adopted an approach that is potentially capable of detecting even components consisting of very short repetitive sequences (≤ 20 base pairs), which could be sufficiently long to serve as regulatory elements within a very small genome. I found that the *Aspergillus* genome most probably contains enough repetitive DNA to transcribe only rRNA, tRNA, and, perhaps, a few mRNA's.

High-molecular-weight DNA was prepared from purified nuclei isolated from stationary phase cultures of *A. nidulans* (*19*) and was found to have the following physical parameters: (i) buoyant density in CsCl, 1.709 g/ml; (ii) melting temperature ($T_m$) in 0.12*M* sodium phosphate buffer (*20*), 90°C; (iii) hyperchromicity as the percentage of initial absorbancy at 260 nm ($A_{260\ nm}$), 38 percent; and (iv) modal single-strand fragment length, ≥ 20,000 nucleotides. Equilibrium banding of varying amounts of nuclear DNA failed to reveal any satellite components.

DNA fragments having a modal single-strand fragment length of 500 nucleotides were reassociated at 60°C in 0.12*M* phosphate buffer (Fig. 1A). Least-squares analysis of the data (*20, 21*) indicated the presence of only one kinetic component reassociating with a second-order rate constant ($K$) of 0.042 $M^{-1}$ $sec^{-1}$. Less than 1 percent of the DNA was bound to hydroxyapatite at the lowest $C_0t$ used ($10^{-2}$), showing that the *A. nidulans* genome contains very few fold-back sequences. Using the reassociation rate constant of *Escherichia coli* DNA as a reference (*3*), I calculate the genome size of *A. nidulans* to be $2.6 \times 10^7$ base pairs or 0.028 pg per haploid nucleus, a value in good agreement with that obtained by Bainbridge (*22*) using a colorimetric assay of conidial extracts (0.022 pg).

The data indicate that *Aspergillus* DNA has very little reiterated DNA (Fig. 1A); however, there is too much scatter to assess accurately the possible contribution of a small repetitive component. Furthermore, a reassociated repetitive component consisting of either very short or highly mismatched sequences might be unstable at the 60°C, 0.18*M* $Na^+$ criterion used (30°C below the $T_m$) (*23*). I therefore reassociated radioactively labeled DNA in the presence of a large excess of unlabeled, 500-nucleotide, nuclear DNA at two criteria (60°C, 0.18*M* $Na^+$; 50°C, 0.21*M* $Na^+$). The results are shown in Fig. 1B. As expected, the labeled DNA reassociated at the 60°C, 0.18*M* $Na^+$ criterion with kinetics similar to unlabeled DNA (compare to Fig. 1A). Least-squares analysis revealed the presence of only one kinetic component with a $K$ of 0.051 $M^{-1}$ $sec^{-1}$. Since the single-strand length of the labeled DNA was 1.16 times that of the unlabeled "driver" DNA, this rate was in good agreement with that derived from Fig. 1A ($1.16 \times 0.042 = 0.049$) (*20*). Reassociation of the same labeled DNA at the lower criterion resulted in a substantially reduced rate constant (0.016 $M^{-1}$ $sec^{-1}$), which is expected at 41°C below the $T_m$ (*20*), but was still well fit by a single second-order kinetic component. Thus, even low criterion reassociation failed to reveal any repetitive component. Since hydroxyapatite might be incapable of binding very short DNA duplexes, I also assayed reassociation at low $C_0t$ values (0.1 and 1.0; 60°C, 0.18*M* $Na^+$) by treating the reaction mixtures with the single-strand specific nuclease S1 and precipitating the nuclease-resistant duplexes with trichloroacetic acid (*9*). At $C_0t$ of 0.1, 1.0 percent of the labeled DNA was resistant to S1 nuclease as compared to 0.4 percent bound to hydroxyapatite. At $C_0t$ of 1.0, 4.2 percent of the DNA was precipitated by trichloroacetic acid as opposed to 6.4 percent bound to hydroxyapatite. Thus, as before, no significant fraction of repetitive DNA was detected.

In order to ascertain whether *A. nidulans* DNA contained any reiterated sequences, I reassociated radioactively labeled DNA, in an excess of unlabeled DNA, to $C_0t$ of 1. The duplex-containing molecules were recovered by hydroxyapatite chromatography and reassociated in a large excess of unfractionated, 500-nucleotide, nuclear DNA. The labeled DNA reassociated with kinetics consistent with the presence of two components (Fig. 2). One of these, representing 70 percent of the labeled DNA, reassociated with a $K$ of 0.06 $M^{-1}$ $sec^{-1}$, and represented single-copy DNA. The re-


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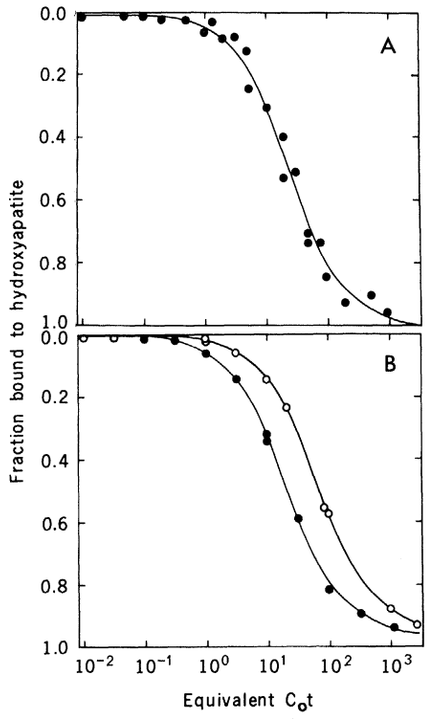
maining 30 percent reassociated with a $K$ of 3.5 $M^{-1}$ sec$^{-1}$ and therefore represented a reiterated component. From the ratios of the rate constants I calculate that this component is repeated approximately 60 times per haploid genome. I further calculate that it has a complexity of approximately 11,000 base pairs and represents 2 to 3 percent of the genome (*24*).

The complexity and reiteration frequency of the repetitive component suggested that it might represent the sequences coding for rRNA. To examine this possibility, I hybridized unfractionated, labeled DNA to a 4000-fold excess of *A. nidulans* rRNA to an RNA $C_0t$ ($R_0t$) of 0.1 (DNA $C_0t$, 2.5 × $10^{-5}$) and estimated the hybrid content of the reactions by S1 nuclease treatment and trichloroacetic acid precipitation. Parallel reactions containing no rRNA were used as controls. Only 0.7 percent of the DNA hybridized with the RNA. If asymmetric transcription is assumed, this equates to 1.4 percent of the genome or 3.6 × $10^5$ base pairs. Since the 25*S* and 18*S* rRNA together contain approximately 5700 nucleotides, this value is equivalent to 63 copies per haploid genome, which is essentially identical to the reiteration frequency of the repetitive component revealed by DNA-DNA reassociation. If it is assumed that the rRNA in *A. nidulans* is derived from a larger precursor molecule, then the sequences coding for this precursor could account for most of the reiterated DNA observed. Minor mass components, such as the genes for tRNA or histones, would probably not be detected in these experiments.



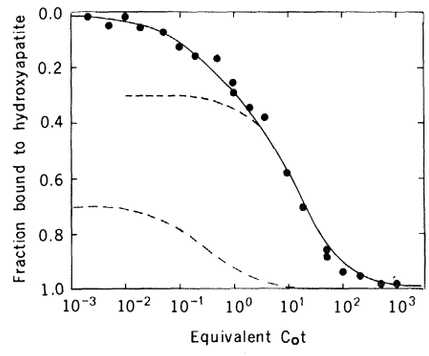




Fig. 1 (left). Reassociation of unfractionated nuclear DNA. *Aspergillus nidulans* (Fungal Genetic Stock Center, No. 4) conidia were inoculated into a medium containing 0.2 percent yeast extract and 0.5 percent D-glucose and grown with vigorous aeration at 37°C to stationary phase. DNA was extracted from purified nuclei which were isolated by a modification of the procedure of Gealt *et al.* (*19*). The DNA, resuspended in 10 m*M* sodium phosphate buffer and 1 m*M* EDTA, was sheared to an average single-strand size of 500 nucleotides in a VirTis 60 homogenizer (*5, 20*) or labeled in vitro with *Escherichia coli* DNA polymerase I (*25*). The modal single-strand fragment lengths were determined by alkaline band sedimentation in an analytical ultracentrifuge (*26*) or by alkaline sucrose density gradient centrifugation (*12*). Reassociation analysis was performed essentially according to Britten *et al.* (*20*) and has been described in detail (*3, 5, 8*). Chromatography on hydroxyapatite was used to separate duplex-containing and single-stranded DNA fragments. (A) Samples of unlabeled DNA were denatured and incubated to various equivalent $C_0t$ values (moles of nucleotides per liter times seconds). The fraction of DNA that was bound or unbound was determined by measuring the $A_{260\ nm}$. The curve shown represents the least-squares best fit of the data with one second-order kinetic component. The parameters describing this solution are as follows: 0.9 percent bound at $C_0t$ 0.01; $K$, 0.042 $M^{-1}$ sec$^{-1}$; root mean square of error (r.m.s.), 2.6 percent. (B) Radioactively labeled DNA having a modal single-strand size of 580 nucleotides was mixed with a large excess of unlabeled 500-nucleotide DNA. Samples were denatured and incubated to various $C_0t$ values at either 60°C, in 0.12*M* phosphate buffer (●), or at 50°C, in 0.14*M* phosphate buffer (○). The fraction of DNA that was bound or unbound was determined by scintillation counting. The curves shown represent the least-squares best fit of the data with one second-order kinetic component for each data set. The solutions were as follows: at 60°C, 0.6 percent bound at $C_0t$ 0.1; $K$, 0.051 $M^{-1}$ sec$^{-1}$; r.m.s., 1.2 percent; at 50°C, 0.4 percent bound at $C_0t$ 0.01; $K$, 0.016; r.m.s., 0.9 percent. Fig. 2 (right). Reassociation of DNA enriched in repetitive sequences. DNA was labeled in vitro (*25*) to a specific radioactivity of 4 × $10^7$ cpm/μg. Fold-back sequences were removed by low $C_0t$ incubation followed by hydroxyapatite fractionation (*8*), and the labeled DNA, having a single-strand fragment length of 580 nucleotides, was reassociated to $C_0t$ of 1 in an excess (100-fold) of unlabeled, 500-nucleotide, nuclear DNA. The duplex-containing molecules (6.2 percent) were recovered by hydroxyapatite chromatography and concentrated by centrifugation to > 5*S* in the ultracentrifuge. The labeled DNA, enriched in putative repetitive sequences, had a single-strand fragment size of 685 nucleotides. This DNA was mixed with a 200,000-fold excess of unfractionated nuclear DNA. Samples were denatured, incubated, and analyzed (see legend to Fig. 1). The solid curve shows the least-squares best fit of the data with two second-order kinetic components, individually represented by the dashed curves. This solution was as follows: 30 percent with a $K$ of 3.5 $M^{-1}$ sec$^{-1}$; 70 percent with a $K$ of 0.06 $M^{-1}$ sec$^{-1}$; r.m.s., 2.6 percent. The predicted $K$ for single-copy DNA having a single-strand fragment length of 685 is 0.058 $M^{-1}$ sec$^{-1}$ [(685/500) × 0.042] (*20*).

In summary, I have found that approximately 97 to 98 percent of the genome of the euascomycete *A. nidulans* consists of DNA sequences present once per haploid genome. The remaining 2 to 3 percent consists of sequences repeated an average of 60 times, with a complexity of only 11,000 base pairs, and, most probably comprises the genes for rRNA. Experiments that were designed to detect very short repetitive sequences did not do so. Thus, it is likely that the genome of *A. nidulans* does not contain repetitive sequences interspersed among its structural genes although the possibility remains that very short repetitive sequences are present but were not detected. A similar situation may exist in the nuclear DNA of the hemiascomycete *Saccharomyces* (yeast) (*7, 17*). If this is the case, it may represent the typical pattern for the Ascomycetidae.

As was pointed out above, the fungi share many properties with higher eukaryotes. My study, as well as several others (*5, 7, 8, 17*), however, suggest that the fungi may differ significantly from more advanced forms in terms of the organization of their DNA and the processes by which they regulate gene expression. Specifically, the observations that the Ascomycetidae coordinately regulate gene sets, do not normally possess operons (*16*), and apparently do not contain interspersed reiterated DNA sequences suggest that current models [for example, (*13, 15*)] for gene regulation may not apply to these organisms.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23. The effect of fragment size on thermal stability can be expressed as $\Delta T_m = B/L$ where $\Delta T_m$ is the difference in $T_m$ of long DNA fragments and that of fragments of some length, $L$, and $B$ is a proportionality constant numerically equal to 650 at 0.18*M* $Na^+$ and 720 at 0.21 *M* $Na^+$ (*20*). Using this expression for the two criteria in Fig. 1B, I calculate that the minimum length for a stable duplex would be 22 base pairs at 60°C and 0.18*M* $Na^+$; and 18 base pairs at 50°C and 0.21*M* $Na^+$.
24. The complexity and genomic content of the repetitive component were calculated as follows: when the labeled DNA was reassociated to $C_0t$ of 1 during the enrichment procedure, 6.2 percent bound to hydroxyapatite. Of this, 30 percent represented the repetitive component and reassociated with a $K$ of 3.5 $M^{-1}$ $sec^{-1}$. This is equivalent to 1.9 percent of the total DNA (0.3 × 6.2 percent). However, at $C_0t$ of 1, only 78 percent of the repetitive component would have reassociated {100 × [1 − 1/( + 3.5)]}. Therefore, the actual representation of the repetitive component in the genome is 2.4 percent (1.9/0.78). This value has been rounded to 2 to 3 percent in the text because of the probability of some inaccuracies in the data. The complexity of this component was then calculated as follows: $K_{pure} = K/0.024 = 146\ M^{-1}$ $sec^{-1}$ (*20*). The complexity of the unique component is $2.6 \times 10^7$ base pairs (Fig. 1), and the $K$ for this tracer was 0.06 $M^{-1}$ $sec^{-1}$. Therefore, the complexity of the repeated component is approximately 11,000 base pairs [(0.06/146) ($2.6 \times 10^7$)].
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# Calcium-Induced Contraction of the Rhizoplast of a Quadriflagellate Green Alga


Abstract. *The rhizoplast of* Platymonas subcordiformis *is a contractile organelle. Cyclic contraction and extension are induced by incubating the organism in solutions containing calcium and adenosine triphosphate. Rhizoplast contraction is functionally linked to flagellar activity.*


Striated fibrous roots occur in association with the flagellar and ciliary apparatus of many eukaryotic cells (*1*). Striated roots are thought to be anchoring structures that absorb stress generated by flagellar or ciliary movement (*1*, *2*), or to represent elements of a signal-transmitting network in certain specialized cells (*3*, *4*). The rhizoplast, a striated fibrous root of the green alga *Platymonas subcordiformis* Hazen, is a massive structure that shows a striking resemblance to myofibrils (Fig. 1A), which has prompted speculation by some authors that it may possess muscular or contractile properties (*5*, *6*). In this report, we present evidence that suggests that the rhizoplast of *Platymonas* is a contractile organelle. We describe conditions under which the rhizoplast can be induced to undergo extraordinary contraction and subsequent extension and propose a functional relationship of the rhizoplast to flagellar locomotion.

When *Platymonas* is prepared for electron microscopy, using standard techniques (*7*), the rhizoplast appears extended as shown in Fig. 1A. A rhizoplast is firmly attached (*8*) to a pair of basal bodies at the anterior end of the quadriflagellate cell. A second rhizoplast, out of the plane of section in Fig. 1A, is attached in a similar fashion to the other pair of basal bodies. Both rhizoplasts extend from the flagellar pit region, pass by and are usually appressed to the nucleus, and terminate posteriorly in a ribosome-free, filamentous network adjacent to the plasmalemma.

Contraction is illustrated by comparison of Fig. 1, A to C. Contraction can be induced and sustained through preparation for electron microscopy by the addition of $CaCl_2$ to the medium and fixative at concentrations of 0.1 to 5 m*M*. Under these conditions all rhizoplast profiles demonstrate the organelle in the contracted state. Calcium ions appear to be directly involved in triggering the contractile process (*9*) and have been demonstrated cytochemically in the contracted but not in the extended rhizoplast

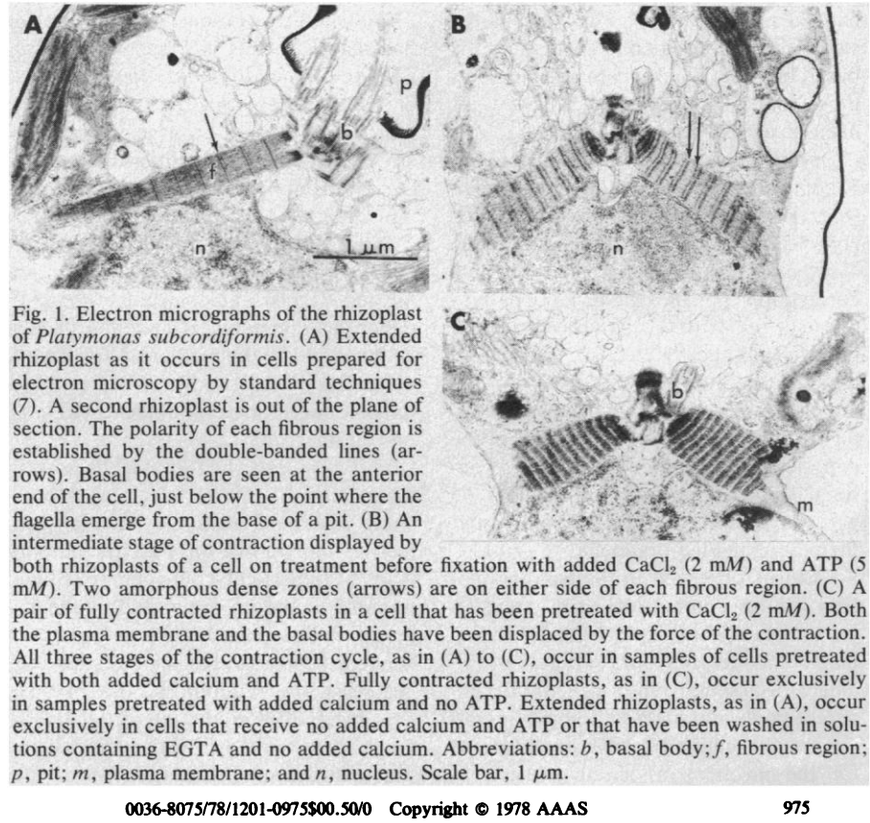


Fig. 1. Electron micrographs of the rhizoplast of *Platymonas subcordiformis*. (A) Extended rhizoplast as it occurs in cells prepared for electron microscopy by standard techniques (*7*). A second rhizoplast is out of the plane of section. The polarity of each fibrous region is established by the double-banded lines (arrows). Basal bodies are seen at the anterior end of the cell, just below the point where the flagella emerge from the base of a pit. (B) An intermediate stage of contraction displayed by both rhizoplasts of a cell on treatment before fixation with added $CaCl_2$ (2 m*M*) and ATP (5 m*M*). Two amorphous dense zones (arrows) are on either side of each fibrous region. (C) A pair of fully contracted rhizoplasts in a cell that has been pretreated with $CaCl_2$ (2 m*M*). Both the plasma membrane and the basal bodies have been displaced by the force of the contraction. All three stages of the contraction cycle, as in (A) to (C), occur in samples of cells pretreated with both added calcium and ATP. Fully contracted rhizoplasts, as in (C), occur exclusively in samples pretreated with added calcium and no ATP. Extended rhizoplasts, as in (A), occur exclusively in cells that receive no added calcium and ATP or that have been washed in solutions containing EGTA and no added calcium. Abbreviations: *b*, basal body; *f*, fibrous region; *p*, pit; *m*, plasma membrane; and *n*, nucleus. Scale bar, 1 μm.



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