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A Chlamydomonas Genomic Library in Yeast Artificial Chromosomes

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Manuscript received January 12, 1995
Accepted for publication June 6, 1995

ABSTRACT

We have constructed and characterized a *Chlamydomonas reinhardtii* total genomic library in yeast artificial chromosomes (YACs). The library contains 7500 clones with inserts ranging in size from 100–200 kb. The representation of the library was assessed by screening one-third of it with a probe derived from the dispersed repeat, *Gulliver,* which occurs ~13 times in the genome. At least 10 of these *Gulliver* loci were isolated within 15 independent YACs. Two of these YACs encompass the *Gulliver* element designated G, which was reported to map to the *uni* linkage group (ULG). The end clones of these two YACs have been genetically mapped by RFLP analysis in an interspecific cross and thereby shown to be closely linked to the *APM* locus on the ULG. A third *uni*-specific YAC has also been isolated and its ends have been mapped by RFLP analysis. Genetic and RFLP analysis of these and other YACs indicates that the frequency of chimeric YACs in the library is very low. The library was constructed in a second generation vector that enables plasmid rescue of YAC end clones as well as copy number amplification of artificial chromosomes. We provide evidence that amplification of intact YACs requires a *rad1:rad52* yeast strain.

THE unicellular alga Chlamydomonas has proved to be an effective experimental model for microtubule-based systems (HARRIS 1989). For example, the flagellar axonemes of Chlamydomonas share their elaborate structure with the axonemes of animal cells but are amenable to study with a combined genetic, biochemical, ultrastructural, and molecular approach beyond that which is possible in an animal model. Our interest in microtubule-based systems is focused on basal bodies and centrioles and their roles in the assembly of complex structures such as the eukaryotic flagellum. We have taken a molecular genetic approach based on the study of one of the linkage groups in Chlamydomonas that bears a cluster of mutants that appear to specifically affect the process of basal body and flagellar development (RAMANIS and LUCK 1986). One group of mutants found on the so called *uni* linkage group (or LG XIX) (JOHNSON and DUTCHER 1991) are temperature sensitive for flagellar assembly. We have successfully cloned the gene corresponding to one of these *fla* mutants and shown that it encodes a kinesin homologous protein that we have proposed is directly involved in flagellar assembly as a transporter molecule (WALTHER *et al.* 1994). Our goal is to characterize additional *uni*-linked mutant genes with a view to exploring their functions in basal body and flagellar morphogenesis.

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Characterization of the *uni* chromosome revealed that it is six to nine megabases in size, making it a suitable candidate for analysis using yeast artificial chromosomes (HALL *et al.* 1989). In our case this approach seemed especially apt because the large insert size these vectors can propagate enables the efficient chromosomal walking and contig building required to encompass the clustered markers we are interested in. We also realized that a total Chlamydomonas genomic library in YACs might find broader utility in the research community for those concentrating on microtubule-based systems as well as for those working with other model systems like photosynthesis (HARRIS 1989). Indeed, the numerous molecular markers that have been mapped throughout the Chlamydomonas genome provide points to initiate analysis of proximal genetic loci using YACs (RANUM *et al.* 1988).

Cloning large DNA fragments in yeast artificial chromosomes is a method that was developed in 1987 and it has since been used successfully for cloning a variety of plant and animal genomes (BURKE *et al.* 1987; GRILL and SOMERVILLE 1991; LARIN *et al.* 1993). More recently, second generation YAC vectors have been developed that facilitate the recovery of endclones and/or the generation of large quantities of DNA by copy number amplification of the YAC in the yeast host (TRAVER *et al.* 1989; SMITH *et al.* 1990; SHERO *et al.* 1991).

This paper describes the construction of a Chlamydomonas genomic library in yeast artificial chromosomes using a second generation vector (SMITH *et al.* 1990). Preliminary characterization of the library indicates that (1) artificial chromosomes containing DNA both from the *uni* chromosome as well as other chromo-

somes can be stably propagated in yeast, (2) the library contains an excellent representation of the Chlamydomonas genome, (3) the vector pCGS966 allows straightforward recovery of plasmids corresponding to both ends of a YAC for genetic mapping and physical walking, and (4) intact Chlamydomonas YACs can be amplified up to 10-fold in a *rad1:rad52* background. This library enables the coupling of genetic mapping and gene identification by positional cloning as in the larger mammalian genome projects. In addition it provides a basis for the identification of mutant loci by direct complementation of mutant phenotypes with amplified wild-type artificial chromosomes (M. VASHISHTHA, G. SEGIL, J. L. HALL, unpublished data).

MATERIALS AND METHODS

Yeast strains: AB1380, (ATCC 20843) *MATa, ura3, trp1, ade2-1, can1-100, lys2-1, his5,* [Ψ^+], were obtained from the ATCC (Rockville, MD). CGY2570, (ATCC 90435)- *MATa, GAL+, ura3, trp1, ade2-1, lys2, his3, leu2,* [Ψ^+] and CGY2897, *MATa, GAL+, ura3, trp1, lys2, his3, rad1::ADE2, rad52::LEU2,* [Ψ^+] were provided by Doug Smith (Genome Therapeutics, Waltham, MA).

Preparation of genomic DNA: The cell wall-less strain of *Chlamydomonas reinhardtii* CW15.227.3C- (obtained from the Chlamydomonas stock collection at Duke University) was grown on R plates containing 0.2 M mannitol (HARRIS 1989). Cells were collected from the plates, washed once in R medium containing 0.4 M mannitol and embedded in low melting temperature agarose blocks at a final concentration of 4×10^8 cells/ml. The blocks were treated to two changes of EDTA-sarkosyl-proteinase K (ESP) for a total of 48 hr at 50° (SMITH *et al.* 1988). Before restriction digestion the blocks were washed extensively in 10 mM Tris-HCl-1 mM EDTA pH 8.0.

Partial *EcoRI* digestion and size fractionation of DNA: Partial digests of the embedded DNA were prepared as described (LARIN *et al.* 1991) except that a combination of 40 units of *Eco*RI methylase and 6-20 units of *Eco*RI restriction enzyme were used, and the incubation time was 90 min. The blocks were subjected to field inversion gel electrophoresis in a 1% low melting temperature agarose gel in 0.5× TBE (CARLE *et al.* 1986). Intervals were 0.2 sec forward and 0.14 sec reverse ramping to 2.1 sec forward and 7.2 sec reverse in 20 steps. Electrophoresis was performed in a BRL HB-4 horizontal system for 11 hr and 45 min at 340 V and 8°. Under these conditions DNA fragments ≥ 100 kb were focused into a single band that was cut out of the gel and prepared for ligation as described (LARIN *et al.* 1991).

Vector preparation and ligation: The YAC vector pCGS966 (SMITH *et al.* 1990) was digested to completion with *Eco*RI and *Bam*HI, treated with alkaline phosphatase, and tested for its ability to ligate to *Eco*RI DNA fragments. Ligation of vector and *C. reinhardtii* DNA was performed according to LARIN *et al.* (1991). After ligation the DNA was refocused by field inversion gel electrophoresis as described here and then cut out of the gel to be used for transformations.

Yeast transformation, colony collection, and filter preparation: High frequency yeast spheroplast transformation was obtained using the protocol of MCCORMICK *et al.* (1990). YAC libraries were generated by transformation of yeast strain AB1380 and plating on either *ura*– or *ura*– *trp*– plates. Individual colonies were picked into microtiter plates containing *ura*– or *ura*– *trp*– medium and grown up for two nights at 30°; replica filters were made as described (BURKE *et al.* 1991).

YAC library screens: Approximately one-third of the library (2500 clones) was screened by colony hybridization with a 700-bp *Eco*RI/*Bgl*I fragment derived from *Gulliver* H (kindly provided by P. FERRIS, Washington University, St. Louis, MO). This fragment hybridizes to each element of the family (JOHNSON and DUTCHER 1991). Positive colonies were grown up in *ura-trp-* medium and analyzed as described in the RESULTS section. YAC B was isolated by screening another third of the library with a subfragment from λ19, one of our original clones of the *uni* chromosome that maps between *UNI* and *FLA9* (HALL *et al.* 1989).

YAC amplification: Amplifications were carried out as described by SMITH *et al.* (1992) using a range of sulfanilamide (1-10 mg/ml) and methotrextrate (10-50 μg/ml) concentrations. Our standard conditions are 2 mg/ml sulfanilamide and 20 μg/μl methotrextrate. Cells were inoculated into amplification medium at a concentration of 1×10^5/ml and grown for 5-10 days at 30° depending on the growth rate.

End cloning and genetic mapping: *Xho*I and *Nde*I restriction digests were set up using high quality genomic DNA prepared from the strain containing the YAC (15-20 μg in a total reaction volume of 100 μl). After digestion and heat inactivation 15 μl of each digest was selfligated in a 20-μl volume. The reactions were ethanol precipitated, rinsed in 70% ethanol and resuspended in 5 μl water. One to two microliters were used to electroporate JS-4 bacterial cells that were plated out on ampicillin (*Nde*I) or kanamycin (*Xho*I) plates.

Genetic tetrad analysis was carried out using standard techniques as described by HARRIS 1989. For mapping the ends of YACs 100A and 116A, DNA was prepared from 35 tetrads from a cross of *C. smithii* × *C. reinhardtii* (*apm, uni*) and the segregation of restriction fragment length polymorphisms (RFLPs) for each of the endclones was scored by Southern analysis. See Figure 3 and legend. For mapping the ends of YAC B, the same method was used except that the genetic cross was *C. smithii* × *C. reinhardtii* (*apm, fla10-1, pf10*) and 17 tetrads were analyzed.

RESULTS

Construction of the library: The vector pCGS966 was selected for two reasons. First, it has bacterial origins of replication and selectable markers on both ends, which enable the rescue of an *Escherichia coli* plasmid corresponding to either end. Second, pCGS966 has a conditional centromere and a heterologous thymidine kinase gene enabling strong selective pressure for amplification of artificial chromosomes (SMITH *et al.* 1990).

The actual construction of the library was based on the methods described by LARIN *et al.* (1991). Genomic DNA fragments in the 100-200 kb size range were derived by adjusting the competing activities of *Eco*RI restriction enzyme and *Eco*RI methylase. Before ligation to the vector, the fragments were size fractionated by field inversion gel electrophoresis (CARLE *et al.* 1986), using conditions that concentrate DNA fragments ≥ 100 kb. After ligation the products were refocused using the same conditions to remove unligated vector and were used to transform strain AB1380 using the high frequency protocol of MCCORMICK *et al.* (1990). We found that inclusion of polyamines in the buffers used for preparation and cloning of the high molecular

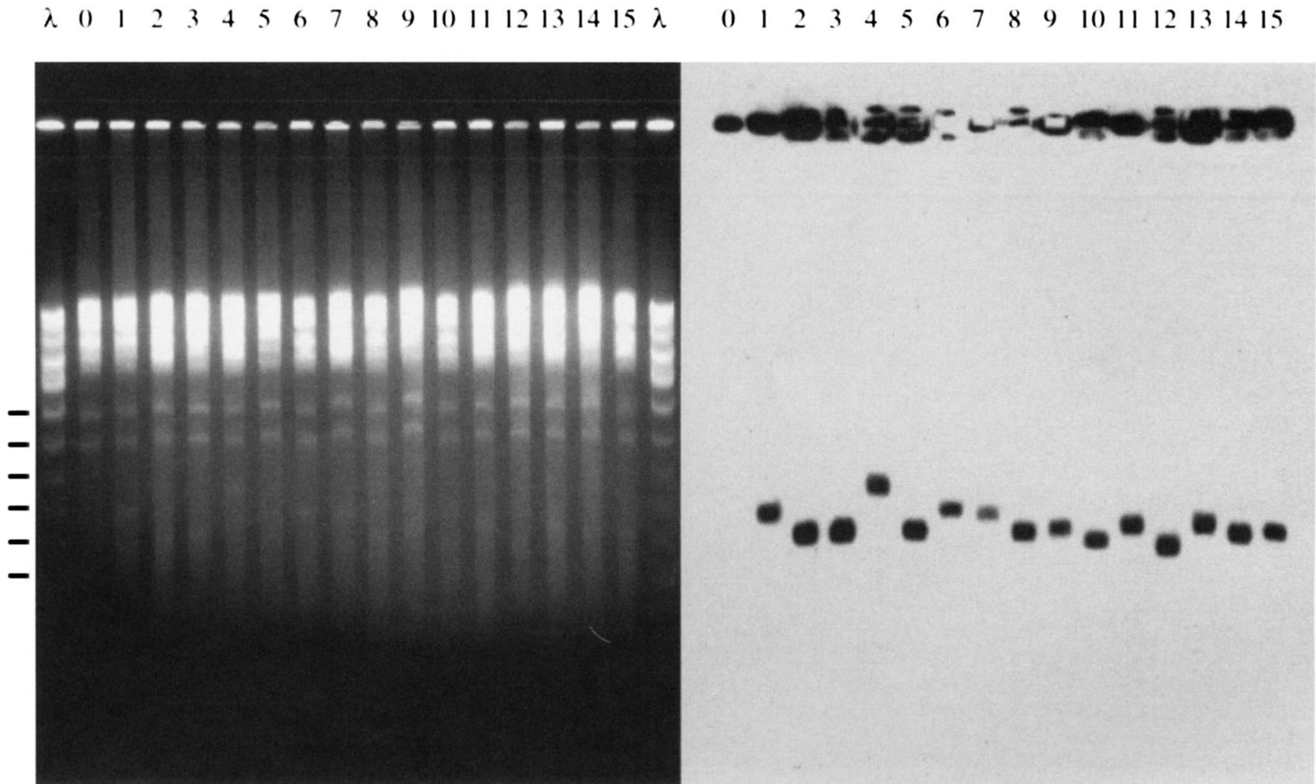

FIGURE 1.—Field inversion gel electrophoresis showing YACs identified with a *Gulliver*-specific probe. At left, the ethidium bromide-stained gel. Bars on the side mark the positions of λ concatemers representing ~50, 100, 150, 200, 250 and 300 kb. At right, a Southern blot of the gel hybridized with pBluescript that detects the YACs in lanes 1–15. Lane 0 contains chromosomes from untransformed AB1380, the strain used to make the library.

weight DNA did prevent degradation as reported (LARIN *et al.* 1991). However, we deliberately omitted polyamines from the transformation step so as not to promote the transformation of very large YACs that could represent chimeras as a result of coligation.

Characterization of the library: To determine whether our library is representative of the Chlamydomonas genome, we screened approximately one-third of it with a dispersed low copy number repeat named *Gulliver* (FERRIS 1989). This 12-kb element is present in ~13 copies in the genome of the CW15.227.3C- strain from which the library was derived. Although *Gulliver* is a transposon similar to those found in higher plants, it appears to transpose at a very low rate so it is also quite useful as a genetic marker. Indeed two copies of *Gulliver* designated G and H have been mapped to the *uni* linkage group (JOHNSON and DUTCHER 1991). Hence, characterization of the library using a *Gulliver*-specific probe could also provide us with additional molecular markers on the *uni* chromosome.

The results of the screen are shown in Figure 1. A total of 15 independent colonies were identified by hybridization with the *Gulliver* probe. Chromosomal DNA from each of these colonies was analyzed by field inversion gel electrophoresis to examine the size range of the YACs. The YACs are visible in the ethidium bromide-stained gel (Figure 1, left), and their sizes can be estimated by comparison with the positions of the lambda concatemers indicated by the bars on the left. The YACs range in size from 100 kb to just under 200 kb. A Southern blot of this gel hybridized with labeled pBluescript (Stratagene) plasmid is shown at right. This plasmid probe hybridizes to both ends of the YAC vector pCGS966 and so confirms that each colony contains only a single artificial chromosome.

One advantage of using *Gulliver* sequences to characterize the library is that the individual loci corresponding to each element can be distinguished by Southern analysis using a single probe and a single restriction enzyme (FERRIS 1989; JOHNSON and DUTCHER 1991). For each locus a *Gulliver*-specific probe hybridizes to a restriction fragment that extends from a common site inside the element to the next adjacent site in the neighboring genomic DNA. As expected for a dispersed repeat these so-called junction fragments differ in size and so uniquely identify each locus. To determine which *Gulliver* loci were represented in each of the 15 YACs, we analyzed their corresponding junction fragments. For this assay, chromosomal DNA from each yeast strain containing *Gulliver* sequences was restricted with *Hin*dIII and run out in a standard agarose gel; the resulting Southern blot was probed with the *Gulliver* sequence used to screen the library. As a control, Chlamydomonas genomic DNA was digested with *Hin*dIII

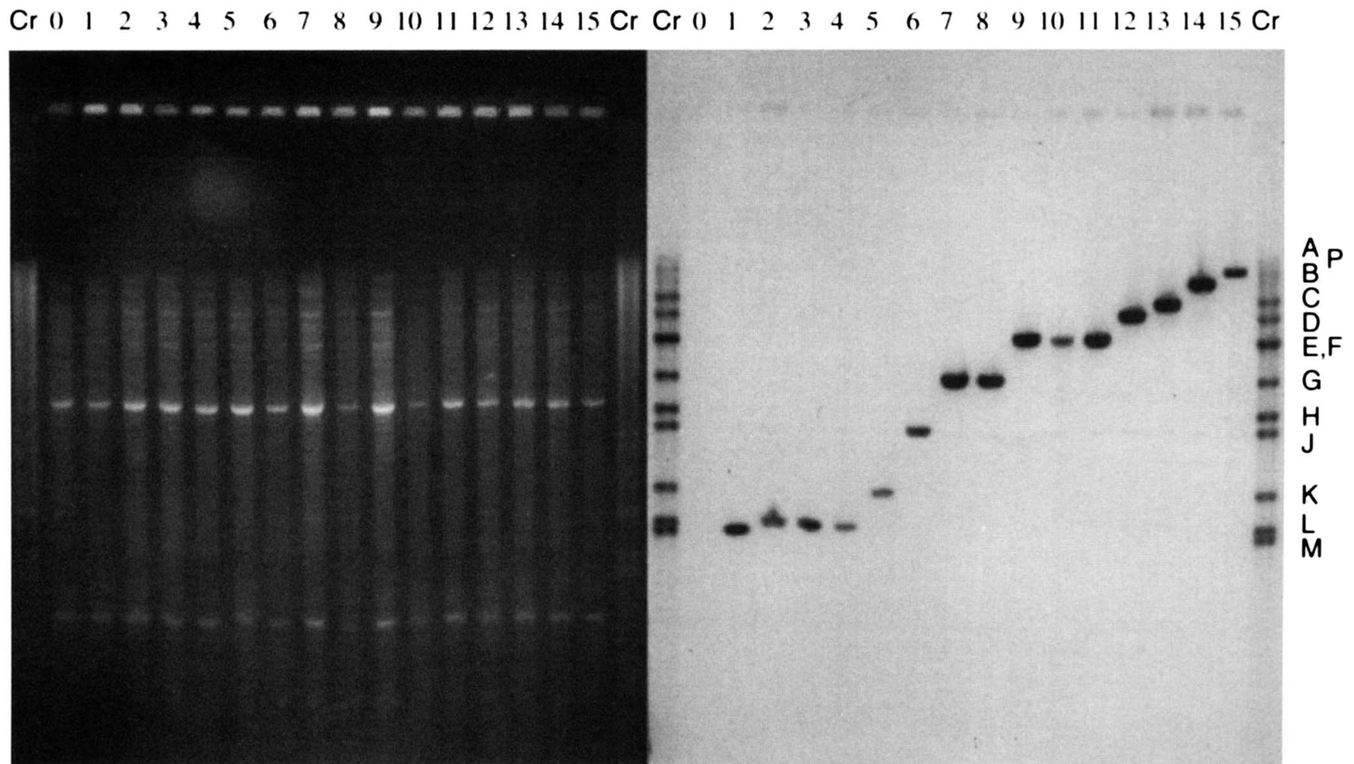

FIGURE 2.—Southern analysis of the 15 *Gulliver* YAC strains. At left, the ethidium bromide-stained gel. At right, a blot of the gel hybridized with the 700-bp *Eco*RI/*Bgl*I *Gulliver* specific probe. Lanes Cr contain digests of *C. reinhardtii* genomic DNA from the cell wall-less strain used to make the YAC library. Lane 0 contains a digest of genomic DNA from untransformed AB1380. Lanes 1–15 contain digests of the *Gulliver* YAC strains. All digests are *Hin*dIII. This result enables identification of the specific *Gulliver* locus recovered in each YAC. The exception is for loci E and F that are not resolved. The three YACS in lanes 9, 10 and 11 correspond to one or both of these loci.

and run out in lanes flanking the yeast digests. The results are shown in Figure 2. In the control lanes marked C.r. there are ~13 bands each corresponding to a *Gulliver* locus. The DNA digest in lane zero comes from untransformed yeast; no hybridization was detected signifying that *Gulliver* sequences are not present in the yeast genome. The lanes marked 1–15 each showed a single band, and each of these corresponds to one of the *Gulliver* loci in the Chlamydomonas genome. Although for most loci a single YAC was isolated, in others two or even three independent YACs belong to a given locus. As described below, we have reason to believe that these are overlapping artificial chromosomes. In all it appear that 10 or 11 of the total of 13 loci have been isolated. Since this screen is based on the use of approximately one-third of our YAC library, the results suggest that additional *Gulliver* loci are likely to be represented in the complete library.

Genetic analysis of two *uni*-linked *Gulliver* YACs: Examination of Figure 2 reveals that one of the *uni*-linked *Gulliver* elements designated G was recovered in two of the YACs isolated in this screen (lanes 7 and 8). This *Gulliver* element has been genetically mapped near the *APM* locus on the *uni* linkage group (JAMES *et al.* 1988; JOHNSON and DUTCHER 1991). We selected these YACs for further analysis. The four end clones of these YACs were obtained by plasmid rescue as described in MATERIALS AND METHODS. Each clone was determined to represent a unique sequence or it was subdivided until a unique fragment was obtained as judged by detection of a single band in genomic Southern blots. Southern analysis was also used to define a RFLP for each clone between the *C. reinhardtii* and *C. smithii* strains. All four of these molecular markers were genetically mapped by scoring the segregation of their corresponding RFLPs in a panel of genomic DNAs derived from an interspecific cross including the genetic markers *apm* and *uni.* The results of this analysis are shown in the diagram in Figure 3 and the relevant tetrad data are shown below the linkage map. The 100A-Xho and 116A-Nde end clones both showed two recombinants relative to *apm,* whereas the 100A-Nde and 116A-Xho end clones both showed three recombinants relative to *apm.* Consistent results were obtained when the endclones were mapped relative to *uni.* As shown in the diagram the two YACs are related as overlaps such that the larger YAC 116A completely encompasses the slightly smaller YAC 100A. Compare the relative size of the YACs in lane 7 (116A) and lane 8 (100A) in Figure 1. This result is consistent with the genetic analysis and was confirmed by blot hybridization experiments showing that both end-clone sequences of 100A are present in 116A. The

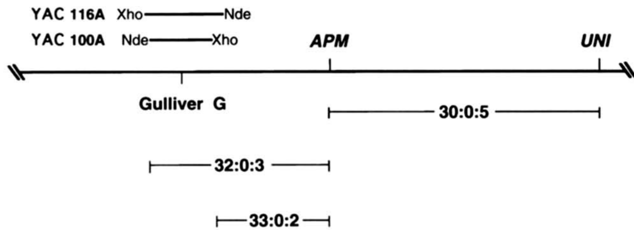

FIGURE 3.—Genetic mapping of two *Gulliver* G YACs closely linked to *APM* and *UNI*. This diagram shows a short segment of the genetic map of the *uni* linkage group. All four end clones of YACs 100A and 116A spanning the *Gulliver* G locus were mapped by RFLP analysis in an interspecifc cross including the markers *apm* and *uni*. 100A Nde and 116A Xho showed three recombinants in 35 tetrads relative to *apm*; 100A Xho and 116A Nde showed two recombinants relative to *apm*. The tetrad data for these intervals are shown below the diagram.

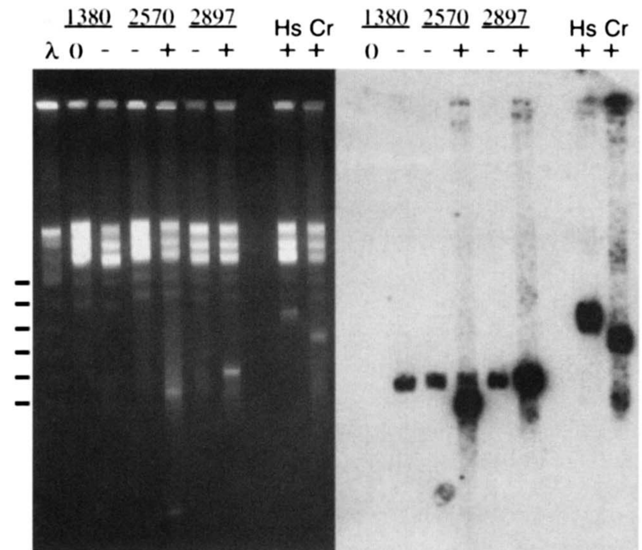

FIGURE 4.—Field inversion gel electrophoresis showing copy number amplification of YACs in strains CGY2570 and CGY2897. At left, the ethidium bromide-stained gel. Bars represent λ size markers. At right, a Southern blot of the gel hybridized with pBluescript. Lane 1380(0) contains chromosomes from the untransformed strain AB1380. Lane 1380(−) is the original YAC B clone, unamplified. Lane 2570(−) is YAC B in CGY2570, unamplified. Lane 2570(+) is YAC B amplified in CGY 2570. Lane 2897(−) is YAC B in CGY2897, unamplified. Lane 2897(+) is YAC B amplified in CGY2897. Amplification of a deleted YAC in CGY2570 can be seen clearly in both the ethidium image as well as in the blot. Amplification of the full-length YAC B in strain CGY2897 is seen by ethidium and blot hybridization. Lane Hs(+) shows amplification of a *Homo sapiens* YAC in CGY2570 (clone 2553, kindly provided by DOUG SMITH). Lane Cr(+) shows amplification of Chlamydomonas *Gulliver* YAC L in CGY2897.

key advantage of finding a recombination event between the loci represented by the YAC end clones is that it orients the YACs on the genetic map and thus reveals which ends are closest to *APM*. We have used the most proximal endclone of 116A to initiate a chromosomal walk to this locus for the purpose of molecular analysis of the *APM* gene.

Finally, as reported by JOHNSON and DUTCHER (1991), the *C. reinhardtii* genomic digests hybridized with the *Gulliver* specific probe shown in Figure 2 can be interpreted as an assay for the relative copy number of sequences corresponding to the various *Gulliver* loci and hence of the chromosomes to which they belong. The intensity of the hybridization signal for *Gulliver* G is indistinguishable from those of other *Gulliver* elements of similar size, meaning that the *uni*-linked element is present in the same copy number as other elements. Thus, in contrast to our original estimation (HALL *et al.* 1989) these data suggest that the *uni* chromosome is present in the same copy number as other chromosomes.

Isolation of a *uni*-linked YAC using a single copy probe: To further characterize the YAC library and to determine the possibility of isolating YACs identified by unique sequence probes, we screened approximately one-third of the library with a probe derived from one of our original molecular markers on the *uni* chromosome designated λ19 (HALL *et al.* 1989). A single positive clone was isolated (YAC B) and subsequently shown to contain a ~100 kb YAC that spans the λ19 sequences. Using the end-cloning strategy outlined for YACs 100A and 116A, both ends of YAC B were mapped by RFLP analysis in an interspecific cross. In this case no recombination was detected between the ends of YAC B or between either of the ends and λ19 (17:0:0; PD:NPD:TT).

Amplification of Chlamydomonas YACs: In the system described by SMITH *et al.* (1992) amplification of artificial chromosomes is made possible by inactivating the centromere by inducing transcription from an adjacent *GAL1* promoter and using the marker thymidine kinase (TK) to select for high copy number of the YAC per cell. To test the feasibility of amplifying Chlamydomonas artificial chromosomes, YAC B was transformed into the *GAL+* strain CGY2570 and amplification was attempted using several different concentrations of methotrexate and sulfanilamide. After amplification, the chromosomes were analyzed by field inversion gel electrophoresis (Figure 4). The ethidium-stained gel is shown on the left and the corresponding blot hybridized with the pBluescript plasmid probe is shown on the right. The lane labeled 1380(−) contains the original YAC B clone isolated from the library. The minus sign indicates that the culture was not amplified. The YAC was transformed into strain CGY2570 and as shown in the lane marked 2570(−), the full size YAC was recovered in this isolate. The lane marked 2570(+) shows the results after amplification of this clone. The full-size YAC B is still present though it is not amplified, whereas a deletion derivative of the YAC has undergone amplification. A similar result was obtained with another isolate of YAC B in CGY2570 as well as with two completely unrelated non-*uni*-linked YACs (data not shown). Nor did lowering the drug concentration change the result: the full length YAC failed to amplify

and was sometimes lost completely whereas a deletion derivative of the YAC was always amplified. To confirm that our conditions did not somehow preclude amplification of any YAC at all, we attempted amplification of a human YAC clone in CGY2570. This YAC was effectively amplified using our standard conditions (Figure 4, lane Hs+). Apparently, these conditions are recombinogenic for the *C. reinhardtii* YACs in strain CGY2570 and they undergo recombination yielding a smaller construct that is amplifiable. To address this problem, we attempted to amplify the YACs in a *GAL+* strain deficient for functions known to be involved in mitotic recombination (*rad1:rad52*) (KLEIN 1988; SCHIESTL and PRAKASH 1988; THOMAS and ROTHSTEIN 1989; FISHMAN-LOBELL and HABER 1992; LING *et al.* 1993). After transferring YAC B into CGY2897 we successfully amplified the full-length YAC by a factor of ten, as seen in Figure 4, lane 2897(+). To confirm that this solution would work for other *C. reinhardtii* YACs, we transformed one of the larger *Gulliver* YACs (Figure 1, lane 4) into CGY2897 and demonstrated amplification of the full-length YAC (Figure 4, lane Cr+). We have since amplified numerous full-length *uni*-linked and non-*uni*-linked YACs in this strain.

DISCUSSION

We have constructed a Chlamydomonas genomic library in yeast artificial chromosomes including ~7500 clones with an average size of ~125 kb. Estimates for the size of the Chlamydomonas nuclear genome range up to 2×10^8 bp, so this library could represent as many as four genome equivalents. Our experience screening the library with the dispersed element *Gulliver* suggests broad representation of the genome: 10 or 11 out of a possible 13 copies of *Gulliver* were isolated from one-third of the library. Screening the library with unique sequence probes has also been successful: at least a single YAC has been identified in the library at more than a dozen different loci (J. L. HALL, unpublished data). Despite these encouraging results we still consider that representation is likely to be incomplete for the following reason. Our strategy for making the library depends on enriching for 100- to 200-kb size fragments using the competing activities of *Eco*RI and *Eco*RI methylase. At the same time no special steps were taken to promote the transformation of fragments >200 kb, meaning that native *Eco*RI restriction fragments much larger than this may be poorly represented. This does predict a gap in coverage corresponding to these fragments that could be remedied by construction of a special large insert *Eco*RI library or by choosing another restriction enzyme to generate high molecular DNA.

Perhaps the most remarkable characteristic of the YACs we have analyzed thus far is the absence of chimerism. This refers to YACs that for any of a number of reasons do not correspond to a contiguous segment of a single chromosome, a phenomenon that is frequently encountered in libraries with very large inserts (SELLERI *et al.* 1992). This observation is based primarily on genetic analysis of YAC endclones mapped as RFLPs in interspecific crosses as in the case of YAC B, 100A, and 116A presented here and for the YACs described by M. VASHISHTHA, G. SEGIL and J. L. HALL (unpublished data). The point is that in all cases the end clones of a YAC are genetically linked to each other, a critical test that a large class of chimeras would fail. Such a low rate of recombinant or chimeric YAC clones is a considerable benefit for chromosome walking as no time is wasted analyzing defective YACs.

We have also performed a series of experiments designed to exploit copy number amplification of YACs cloned in pCGS966. Our initial attempts to amplify in strain CGY2570 resulted in deletion of the YACs and amplification of these deleted structures rather than of the full-length chromosomes. Prompted by evidence for enhanced stability and reduced chimerism of YACs grown in *rad52* and *rad1:rad52* hosts (CHARTIER *et al.* 1992; LING *et al.* 1993), we attempted copy number amplification in a recombination deficient strain. Using the CGY2897 strain, a *rad1:rad52* double mutant, we successfully amplified intact Chlamydomonas YACs and we now use this strain routinely for amplifications. While it would be advantageous to construct a library in CGY2897, we have found that the low transformation efficiency characteristic of this strain would make this an arduous task.

After our success with strain CGY2897 we have not fully characterized the deletions in CGY2570, but several points can be emphasized. First, our observations are not an isolated case associated only with YAC B. Two other unrelated YACs showed similar deletions when amplified in CGY2570. Second, our data are not likely to represent an artifact. Using our reagents we showed that we could amplify a human YAC in CGY2570 and others have successfully amplified a variety of human YACs in this strain. As CGY2570 and CGY2897 are isogenic except for the RAD mutations, our data suggest that one or both of the RAD functions are involved in the deletion events seen after amplification in CGY2570. We have no evidence for a pattern of deletions for any of the YACs we have analyzed and maintained in AB1380 or CGY2570, suggesting that the selective pressure applied for amplifying the YACs triggers the deletion event, perhaps because the deletion product has a growth advantage under these conditions. One pathway that could account for the deletions we have seen involves recombination between tandem repeats that is mediated by RAD1 or RAD52 (NEIL *et al.* 1990). This scenario is consistent with the existence of numerous families of repetitive DNA in the *C. reinhardtii* genome. It is possible that RAD1 and RAD52 act synergistically to yield pop-out deletions between direct repeats in Chlamydomonas YACs. This type of deletion is strongly

suppressed in the *rad1:rad52* double mutant (KLEIN 1988; THOMAS and ROTHSTEIN 1989). Further characterization of the deletions would be necessary to test this hypothesis and to define the recombination pathway involved.

Several factors suggest that our amplifiable YAC library has the potential to become a powerful tool in the molecular genetics of Chlamydomonas. The fact that a single YAC can span an interval that encompasses a recombination event suggests the potential for chromosomal walking. We have demonstrated that amplified wild-type YAC DNA can be used to directly rescue mutant phenotypes providing a relatively straightforward functional assay for the presence of a gene within a YAC (M. VASHISHTHA, G. SEGIL and J. L. HALL, unpublished data). We have also found that the generation of microgram quantities of intact YAC DNA by copy number amplification makes it possible to do a whole range of experiments that would otherwise be extremely difficult to do. For example, we have recently used amplified YAC DNA to make large chromosome-specific probes for *in situ* hybridization experiments (HALL and LUCK 1995).

We wish to thank DOUG SMITH (Genome Therapeutics, Waltham, MA) for the YAC vector and yeast strains as well as for his advice. We are grateful to HANNAH KLEIN (New York University, Department of Biochemistry) and RODNEY ROTHSTEIN (Columbia University College of Physicians and Surgeons, Department of Genetics and Development) for stimulating discussions regarding genetic recombination. This work was supported by grant GM-17132 from the National Institutes of Health.

LITERATURE CITED

BURKE, D. T., G. F. CARLE and M. V. OLSON, 1987 Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science **236:** 806–812.

CARLE, G. F., M. FRANK and M. V. OLSON, 1986 Electrophoretic separations of large DNA molecules by periodic inversion of the electric field. Science **232:** 65–68.

CHARTIER, F. L., J. T. KEER, M. J. SUTCLIFFE, D. A. HENRIQUES, P. MILEHAM *et al.*, 1992 Construction of a mouse yeast artificial chromosome library in a recombination-deficient strain of yeast. Nat. Genet. **1:** 132–136.

FERRIS, P. J., 1989 Characterization of a Chlamydomonas transposon, *Gulliver*, resembling those in higher plants. Genetics **122:** 363–377.

FISHMAN-LOBELL, J., and J. E. HABER, 1992 Removal of nonhomologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene *RAD1*. Science **258:** 480–484.

GRILL, E., and C. R. SOMERVILLE, 1991 Construction and characterization of a yeast artificial chromosome library of Arabidopsis which is suitable for chromosome walking. Mol. Gen. Genet. **226:** 484–491.

HALL, J. L., and D. J. L. LUCK, 1995 Basal body associated DNA: in situ studies in *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA **92:** 5129–5133.

HALL, J. L., Z. RAMANIS, and D. J. LUCK, 1989 Basal body/centriolar DNA: molecular genetic studies in Chlamydomonas. Cell **59:** 121–132.

HARRIS, E. H., 1989 *The Chlamydomonas Sourcebook: A Comprehensive Guide to Biology and Laboratory Use.* Academic Press, New York.

JAMES, S. W., L. P. W. RANUM, C. D. SILFLOW and P. A. LEFEBVRE, 1988 Mutants resistant to anti-microtubule herbicides map to a locus on the *uni* linkage group in *Chalamydomonas reinhardtii.* Genetics **118:** 141–147.

JOHNSON, D. E., and S. K. DUTCHER, 1991 Molecular studies of linkage group XIX of *Chlamydomonas reinhardtii:* evidence against a basal body location. J. Cell Biol. **113:** 339–346.

KLEIN, H. L., 1988 Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *Saccharomyces cerevisiae.* Genetics **120:** 367–377.

LARIN, Z., A. P. MONACO and H. LEHRACH, 1991 Yeast artificial chromosome libraries containing large inserts from mouse and human DNA. Proc. Natl. Acad. Sci. USA **88:** 4123–4127.

LARIN, Z., A. P. MONACO, S. MEIER-EWERT and H. LEHRACH, 1993 Construction and characterization of yeast artificial chromosome libraries from the mouse genome. Methods Enzymol. **225:** 623–637.

LING, L. L., N. S.-F. MA, D. R. SMITH, D. D. MILLER and D. T. MOIR, 1993 Reduced occurrence of chimeric YACs in recombination-deficient hosts. Nucleic Acids Res. **21:** 6045–6046.

MCCORMICK, M. K., J. H. SHERO, C. CONNELLY, S. ANTONARAKIS and P. HIETER, 1990 Methods for cloning large DNA segments as artificial chromosomes in *S. cerevisiae.* Technique **2:** 65–71.

NEIL, D. L., A. VILLASANTE, R. B. FISHER, D. VETRIE, B. COX *et al.*, 1990 Structural instability of human tandemly repeated DNA sequences cloned in yeast artificial chromosome vectors. Nucleic Acids Res. **18:** 1421–1428.

RAMANIS, Z., and D. J. L. LUCK, 1986 Loci affecting flagellar assembly and function map to an unusual linkage group in *Chlamydomonas reinhardtii.* Proc. Natl. Acad. Sci. USA **83:** 423–426.

RANUM, L. P. W., M. D. THOMPSON, J. A. SCLOSS, P. A. LEFEBVRE and C. D. SILFLOW, 1988 Mapping flagellar genes in Chlamydomonas using restriction fragment length polymorphisms. Genetics **120:** 109–122.

SCHIESTL, R. H., and S. PRAKASH, 1988 *RAD1*, an excision repair gene of *Saccharomyces cerevisiae*, is also involved in recombination. Mol. Cell. Biol. **8:** 3619–3626.

SELLERI, L., J. H. EUBANKS, M. GIOVANNINI, G. G. HERMANSON, A. ROMO *et al.*, 1992 Detection and characterization of "chimeric" yeast artificial chromosome clones by fluorescent in situ suppression hybridization. Genomics **14:** 536–541.

SHERO, J. H., M. K. MCCORMICK, S. E. ANTONARAKIS and P. HIETER, 1991 Yeast artificial chromosome vectors for efficient clone manipulation and mapping. Genomics **10:** 505–508.

SMITH, C. L., S. KLCO and C. R. CANTOR, 1988 Pulsed field gel electrophoresis and the technology of large DNA molecules, pp. 41–72 in *Genome Analysis: A Practical Approach*, edited by K. DAVIES. IRL Press, Oxford.

SMITH, D. R., A. P. SMYTH and D. T. MOIR, 1990 Amplification of large artificial chromosomes. Proc. Natl. Acad. Sci. USA **87:** 8242–8246.

SMITH, D. R., A. P. SMYTH and D. T. MOIR, 1992 Copy number amplification of yeast artificial chromosomes. Methods Enzymol. **216:** 603–614.

THOMAS, B. J., and R. ROTHSTEIN, 1989 The genetic control of direct-repeat recombination on saccharomyces: the effect of *rad52* and *rad1* on mitotic recombination at *GAL10*, a transcriptionally regulated gene. Genetics **123:** 725–738.

TRAVER, C. N., S. KLAPHOLZ, R. W. HYMAN and R. W. DAVIS, 1989 Rapid screening of a human genomic library in yeast artificial chromosomes for single-copy sequences. Proc. Natl. Acad. Sci. USA **86:** 5898–5902.

WALTHER, Z., M. VASHISHTHA and J. L. HALL, 1994 The Chlamydomonas *FLA10* gene encodes a novel kinesin-homologous protein. J. Cell Biol. **126:** 175–188.

Communicating editor: S. L. ALLEN