MOLECULAR AND CELLULAR BIOLOGY, Apr. 1988, p. 1469–1473
0270-7306/88/041469-05$02.00/0

Vol. 8, No. 4

An Electrophoretic Karyotype of *Neurospora crassa*

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A molecular karyotype of *Neurospora crassa* was obtained by using an alternating-field gel electrophoresis system which employs contour-clamped homogeneous electric fields. The migration of all seven *N. crassa* chromosomal DNAs was defined, and five of the seven molecules were separated from one another. The estimated sizes of these molecules, based on their migration relative to *Schizosaccharomyces pombe* chromosomal DNA molecules, are 4 to 12.6 megabases. The seven linkage groups were correlated with specific chromosomal DNA bands by hybridizing transfers of contour-clamped homogeneous electric field gels with radioactive probes specific to each linkage group. The mobilities of minichromosomal DNAs generated from translocation strains were also examined. The methods used for preparation of chromosomal DNA molecules and the conditions for their separation should be applicable to other filamentous fungi.

Alternating-field gel electrophoresis technology has advanced rapidly in recent years resulting in the resolution of increasingly larger DNA molecules (5, 19). These advances have allowed the electrophoretic karyotyping of several organisms, including *Saccharomyces cerevisiae* (4, 19), *Schizosaccharomyces pombe* (21, 27), *Candida albicans* (22), and *Trypanosoma brucei* (24). The upper limit of resolution with this technique is continually being extended, although most DNA molecules that have been separated to date are under 2.5 megabases long. Recently, resolution of *S. pombe* chromosomal DNAs has been achieved by using the contour-clamped homogeneous electric field (CHEF) gel system (27) and the pulsed-field gradient gel system (21). The size of the largest of these molecules was estimated to be 7 megabases. This advance significantly increases the number of organisms whose chromosomal DNAs may be electrophoretically resolved.

Neurospora crassa is a filamentous ascomycete with seven linkage groups defined by detailed genetic maps (16). It is an attractive organism for application of the improved resolving power of the CHEF gel system. Based on physical measurements of the *N. crassa* haploid genome size (8, 9) combined with cytological estimates of the relative sizes of the seven chromosomes (12, 20), the sizes of the chromosomal DNAs can be estimated to range from 2.5 to 11 megabases. Separation of the *N. crassa* chromosomal DNAs would facilitate molecular studies of chromosome organization and aid in the cloning of genes which are not selectable.

Using the CHEF gel system and linkage-group-specific probes, we defined the migration of chromosomal DNAs corresponding to all seven linkage groups and separated five of the seven molecules. We also analyzed the migration of the DNAs of four minichromosomes from strains containing translocations. These and other strains containing translocations permit the separation of the DNAs of specific chromosome arms. Rough estimates of chromosomal DNA size based on the migration of the wild-type and minichromosomal DNAs relative to that of the *S. pombe* and *S. cerevisiae* chromosomal DNAs suggest that the genome size is approximately 47 megabases.

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MATERIALS AND METHODS

Strains and plasmids. Chromosome-size DNA molecules were prepared from the following *N. crassa* strains: 74-OR23-1*A* (74*A*) (FGSC 987), *T(IIR→VL)ALS176a* (FGSC 3014), *T(IIL→X;IV;V)AR179a* (FGSC 2596), *T(VIIL→IVR) T54M50a* (FGSC 2467), and *T(IIIL;VR)OY339A* (FGSC 3687). *S. cerevisiae* YNN295 and *S. pombe* wild-type strain 975 (h^+) were used as sources of chromosomal DNA for markers (5, 27).

All plasmids used for probes were grown in *Escherichia coli* JM101 (29). Plasmid DNA was isolated by the alkaline lysis method of Birnboim and Doly (2). Plasmids containing *Neurospora* genes were used as probes of chromosomal DNA blots: pACqa-2A *qa-2* (linkage group VII [LG VII]) (W. Schneider and C. Yanofsky, unpublished data), pAR201 *arg-2* (LG IV) (M. J. Orbach, unpublished data), pNC2 *trp-1* (LG III) (18), pDB1 *trp-3* (LG IIR) (D. Burns and C. Yanofsky, unpublished data) pFB6 *pyr-4* (LG IIL) (3), pBT3 *tub-2* (LG VI) (14), and pNH60 *his-3* (LG I) (10). The *tub-2* locus is also referred to as *Bml* (16). It encodes β-tubulin (14) and was originally defined by mutations responsible for resistance to benomyl. A fragment from cosmid 5:3:F of the pSV50 cosmid library (26) containing the *N. crassa* rDNA repeat unit was used as a probe for LG V.

Preparation of intact chromosomal DNA. *N. crassa* chromosomal DNA was prepared by either of two methods based on procedures currently used for *S. cerevisiae* or *S. pombe* (5, 27). Conidia were harvested and were counted with a hemacytometer; 10^9 conidia were suspended in 150 ml of Vogel minimal medium (25) and grown at 16°C with shaking at 200 rpm for 9 h. Germinated conidia containing germ tubes one to four times the length of a conidium were harvested, pelleted, and washed three times with 50 mM EDTA (pH 8.0). At this point the two procedures diverged. In the liquid spheroplast procedure, the germinated conidia were suspended in 10 ml of 1 M sorbitol–50 mM EDTA–50 mM sodium citrate (pH 5.7) before the addition of 2 ml of a solution of 5 mg of Novozym 234 (Novo Laboratories) per ml in 1 M sorbitol. Digestion by Novozym was done at 30°C with shaking at 100 rpm until the cells were osmotically sensitive. Osmotic sensitivity was assessed by exposing a sample of cells to distilled water and observing their lysis

microscopically. After 4 h of Novozym treatment more than 80% of the cell sample lysed in response to exposure to water. The presence of citrate buffer in the mixture was required to generate osmotically sensitive cells. After Novozym treatment, the cells were pelleted and washed three times with 1 M sorbitol–50 mM EDTA (pH 8.0). The cells were then mixed with molten 1% low-melting-point agarose at 50°C in 1 M sorbitol–50 mM EDTA (pH 8.0) containing 2 mg of proteinase K per ml, such that the final concentration of agarose was 0.6% and the final cell density was 4×10^8/ml. The cell-agarose solution was drawn into a 1-ml tuberculin syringe and gelled by placing the syringe in ice. The end of the syringe was cut off, and the solidified plug was extruded. The agarose plugs were then placed in NDS buffer (0.5 M EDTA [pH 8.0], 10 mM Tris hydrochloride [pH 9.5], 1% *N*-lauroylsarcosine) (19) and incubated for 24 h at 50°C.

In the second method, the agarose spheroplast method, the germinated conidia were mixed with molten 1% low-melting-point agarose in 125 mM EDTA–50 mM sodium citrate (pH 5.7) containing Novozym 234, and the cell-agarose solution was allowed to gel as described above. The conidia and agarose were mixed such that the final agarose concentration was 0.6%, the final cell density was 4×10^8 conidia per ml, and the final Novozym 234 concentration was 2.7 mg/ml. Spheroplasts were generated by placing the agarose plugs in 50 mM sodium citrate (pH 5.7)–0.4 M EDTA (pH 8.0)–7.5% 2-mercaptoethanol and incubating them at either 37 or 45°C for 24 h. The plugs were rinsed three times with 0.4 M EDTA (pH 8.0) after spheroplast generation. To lyse the cells, the plugs were placed in NDS buffer (19) containing 2 mg of proteinase K per ml and incubated for 24 h at 50°C.

After the cell lysis step, plugs prepared by either method were rinsed three times with 50 mM EDTA (pH 8.0) at room temperature. These plugs were then stored at 4°C. The chromosomal DNA in these plugs is stable for at least 3 months.

Gel conditions. CHEF gel electrophoresis was performed as described previously (5, 27) using a modified gel apparatus that has vertical instead of horizontal electrodes and driving electrodes separated by 30 cm. All gels were electrophoresed in 0.5× TBE buffer (Tris-borate, EDTA [11]) that was cooled to 9°C. Agarose gels (0.6%) were formed by pouring 35 ml of molten agarose in 0.5× TBE buffer onto 10.5-cm square glass plates (0.2 cm thick). DNA samples were cut from the agarose plugs (see above) to fit into the gel wells and sealed into the wells with 1% low-melting-point agarose. The voltage, switching intervals, and total times of electrophoresis are described in Results. The gels were then stained with ethidium bromide (0.5 μg/ml) for 20 min and destained in distilled water for 20 min.

Transfer and hybridization conditions. The gels were irradiated for 1 min with UV light (254 nm) to nick the DNA before transfer. The gel was then incubated in 250 ml of 0.5 M NaOH–1.5 M NaCl for 30 min to denature the DNA and neutralized for 30 min in 250 ml of 0.5 M Tris hydrochloride (pH 7.5)–1.5 M NaCl. The DNA was transferred to Genatran 45 membranes (Plasco) by capillary action with 10× SSPE (11) as the buffer. The filters were baked for 2 h at 80°C under vacuum and prehybridized in sealed plastic bags for 3 to 6 h at 42°C in 15 ml of 3× SSPE–50% formamide–100 μg of denatured salmon sperm DNA per ml–5× Denhardt solution (6)–1% sodium dodecyl sulfate (SDS)–5% dextran sulfate. Probes, denatured by boiling for 5 min, were injected into the prehybridization bags, and the hybridization mixtures were incubated at 42°C for 18 to 24 h. The probes were prepared by the random hexamer priming method of Feinberg and Vogelstein (7) by using [α-^{32}P]dCTP (>3,000 Ci/mmol; Amersham Corp.). Probes with specific activities greater than 6×10^8 cpm/μg were used at a concentration of 1×10^6 to 2×10^6 cpm/ml. Filters were washed once in 120 ml of 50% formamide–3× SSPE–0.2% SDS at 42°C for 15 min followed by two 30-min washes in 300 ml of 0.5× SSPE–0.1% SDS at 60°C. The filters were stripped between probings by immersion in 300 ml of boiling 0.5× SSPE–0.1% SDS, followed by a 30-min incubation in the same buffer at 75°C.

RESULTS

To resolve the seven *N. crassa* chromosomal DNAs, we first used CHEF gel conditions suitable for separation of the three *S. pombe* chromosomes (27). Chromosomal DNA samples prepared from wild-type *N. crassa* 74A (details in Materials and Methods) were subjected to electrophoresis at 40 V, using a 60-min switching interval. The *N. crassa* chromosomal DNAs were resolved into four bands (Fig. 1A, lanes 1 and 3). The migration of DNAs corresponding to the seven linkage groups was determined by transferring the DNAs to a nylon membrane, followed by successive hybridizations with ^{32}P-labeled probes representing each linkage group (Fig. 1B). Under these electrophoresis conditions, LG IV and LG III DNAs (probes *arg-2* and *trp-1*, respectively) were separated as unique bands. The DNAs of the two largest chromosomes, LG I and V (probes *his-3* and *rDNA*, respectively), migrated as a single band, as did those from the three smaller chromosomes, LG II, VI, and VII (probes *trp-3*, *tub-2*, and *qa-2*, respectively).

The molecular weight range in which CHEF gel electrophoresis gives maximal resolution can be shifted upward by increasing the switching interval (27). In Fig. 1, the midsized *N. crassa* chromosomal DNAs (LG IV and III) are in this region of maximal resolution. To separate the two largest *N. crassa* chromosomal DNAs, the switching interval was increased from 60 to 99 min. In addition, the voltage was decreased from 40 to 31 V because very large DNA molecules have been found to migrate more reproducibly at low

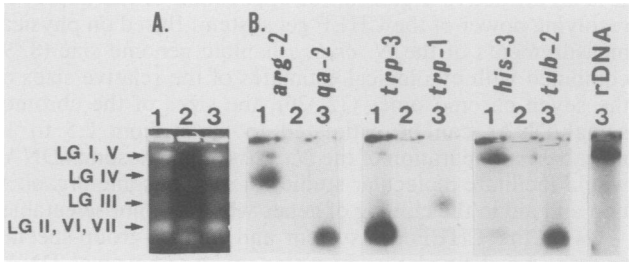

FIG. 1. Separation of *N. crassa* chromosomal DNA on a CHEF gel. (A) An agarose gel electrophoresed for 137 h at 40 V with a 60-min switching interval. The gel was stained with ethidium bromide. Lanes 1 and 3, *N. crassa* samples prepared by the agarose spheroplast method (see the text); lane 2, *S. pombe* chromosomal DNAs as size markers. (B) Hybridization of separated chromosomal DNAs with probes representing each of the seven *N. crassa* linkage groups. After transfer of the DNAs to a nylon membrane, the membrane was cut and probed as shown. The genes used as probes are given above the autoradiograms. The filters were stripped between successive probings. The linkage group of each probe is as follows: *arg-2*, LG IV; *qa-2*, LG VII; *trp-3*, LG II; *trp-1*, LG III; *his-3*, LG I; *tub-2*, LG VI; and *rDNA*, LG V. A summary of the hybridization results (panel B) is shown to the left of panel A.

field strengths (21, 27). Under these conditions, the distance between the LG IV band and the two large chromosomal DNAs was increased, but LG I and V were still unresolved (data not shown). Increasing the switching interval to 140 min and carrying out electrophoresis at 30 V for 168 h did resolve the two largest *N. crassa* molecules (Fig. 2A). Chromosome-specific probes identified the band with the lowest mobility as LG I and the band just below it as LG V (Fig. 2B and 2C). The separation of LG V and LG I DNAs was greatly improved by increasing the switching interval to 180 min and carrying out electrophoresis for 192 h (Fig. 2D).

To separate the three smallest chromosomal DNAs, the switching interval was decreased to 52.5 min from the 60 min used in the experiment for which results are shown in Fig. 1. This lowered the region of maximal resolution to the 3- to 5-megabase range, as judged by the migration of the *S. pombe* chromosomal DNAs (Fig. 3), and separated the three small chromosomal DNAs into two bands. Chromosome-specific probes identified the band with lower mobility as LG II (*trp-3*, Fig. 3), while the band with greater mobility hybridized to probes for LG VI (*tub-2*, Fig. 3) and LG VII (*qa-2*, data not shown). Decreasing the switching interval to 45 min did not resolve the two smallest chromosomal DNAs even though they migrated in the region of highest resolution. We conclude that these two molecules are very similar in size.

Several *N. crassa* strains have translocations that significantly alter the sizes of the normal chromosomes (15). Appropriate strains could be used to achieve separation of LG VI and VII DNAs. For example, in strain *T(VIIL→IVR) T54M50* the LG VII chromosome is replaced by a minichromosome containing sequences of LG VIIR; the left arm of LG VII is attached to LG IV. Chromosomal DNAs from this strain and three other translocation strains were prepared and electrophoresed on CHEF gels (Fig. 3A). Probe analysis

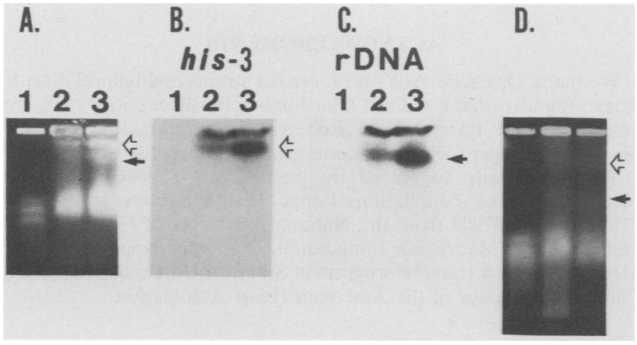

FIG. 2. Separation of the two largest *N. crassa* chromosomal DNAs. (A) An agarose gel electrophoresed for 168 h at 30 V with a 140-min switching interval. The gel was stained with ethidium bromide, and the DNAs were transferred as described for Fig. 1. Lane 1, *S. pombe* chromosomal DNAs as size markers; lanes 2 and 3, *N. crassa* samples prepared by the agarose spheroplast method with 37 and 45°C incubation temperatures, respectively, for the Novozym 234 treatment. The open arrow indicates the lower-mobility band (LG I) in all panels; the solid arrow indicates the band of greater mobility (LG V). (B) DNA transfer probed with the *his-3* gene to detect LG I. (C) Probe of filter (after being stripped) with an *rDNA* fragment to detect LG V. There was significant hybridization to DNA remaining in the wells with both the *his-3* and *rDNA* probes. Also, faint cross-hybridization was detected between the *N. crassa rDNA* probe and the *S. pombe* DNA. (D) An agarose gel electrophoresed for 192 h at 30 V with a 180-min switching interval. The order of samples is the same as that in panel A.

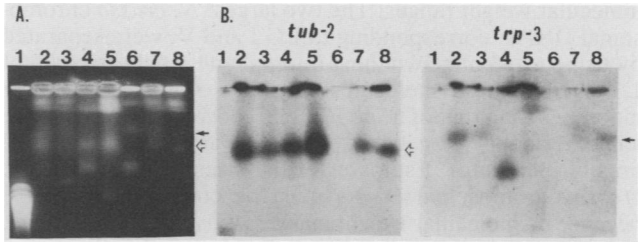

FIG. 3. Electrophoretic analysis of DNAs from *N. crassa* strains containing translocations that generate minichromosomes. (A) An agarose gel electrophoresed for 165 h at 40 V with a 52.5-min switching interval. The gel was stained with ethidium bromide, and the DNAs were transferred to a nylon membrane as described for Fig. 1. Lanes: 1, *S. cerevisiae* YNN 295; 2 to 5, *N. crassa* translocation strains *T(IIIL;VR)OY339A* (lane 2), *T(VIIR→IVR) T54M50a* (lane 3), *T(IIL→X;IV;V)AR179a* (lane 4), and *T(IIR→VL) ALS176a* (lane 5); 6, *S. pombe* 975; 7 and 8, DNAs from *N. crassa* wild-type strain *74A* prepared by the agarose and liquid spheroplast methods, respectively. (B) Filter probed successively with *tub-2* (LG VI) and *trp-3* (LG IIR) DNAs. The filter was stripped between probings. In both panels, the closed arrow indicates the band in strain *74A* corresponding to LG II DNA and the open arrow indicates the band corresponding to LG VI and LG VII DNAs.

of *T(T54M50)* (Fig. 3B, *tub-2*, lane 3) revealed that LG VI DNA migrated as a unique band with the same mobility as that of the LG VI DNA of the wild-type strain *74A* (Fig. 3B, *tub-2*, lanes 7 and 8). The LG VIIR minichromosomal DNA (Fig. 3A, lane 3) migrated with a mobility near that of the largest *S. cerevisiae* molecule (Fig. 3A, lane 1). Presumably, the mobility of the LG IV-LG VIIL chromosomal DNA was lower than that of LG IV (not tested).

All four translocation strains examined have chromosomal DNA bands of lower molecular weight than those from strain *74A* (Fig. 3A). These minichromosomal DNAs have mobilities in the range of those for molecules whose size is known and therefore can be used for estimating the sizes of the larger chromosomal DNAs for which there are no known size markers. Of particular interest are translocation strains *T(IIR→VL)ALS176* and *T(IIL→X;IV;V)AR179*. The minichromosomes which are generated in these two strains (Fig. 3A, lanes 5 and 4, respectively) represent the left and right arms, respectively, of LG II with the LG II centromere. In both strains the translocation breakpoints map near the centromere but on opposite sides, suggesting that they have a relatively small region in common. In *T(AR179)*, the LG IIR minichromosomal DNA (Fig. 3A, lane 4) has a mobility approximately equivalent to that of chromosome III DNA of *S. pombe* (Fig. 3A, lane 6), whose length is about 3 megabases (27). In *T(ALS176)* the IIL minichromosomal DNA (Fig. 3A, lane 5) has a mobility slightly lower than that of *S. cerevisiae* chromosome IV DNA (data not shown), whose length is about 1.6 megabases (13). Addition of these lengths suggests that LG II DNA is about 4.6 megabases long. The migration of LG II DNA relative to that of the 5-megabase *S. pombe* chromosome II DNA (27) is consistent with this size estimate.

DISCUSSION

We prepared chromosome-size DNA molecules from germinated conidia of *N. crassa* and separated these molecules by alternating CHEF gel electrophoresis. By varying the electrophoretic conditions, the window of maximal resolution was positioned to resolve DNA molecules in three

molecular weight ranges. The two largest *N. crassa* chromosomal DNAs, corresponding to LG I and V, were separated by using a 140-min switching interval and a field strength of 1.3 V/cm. These molecules exhibited significantly lower mobilities than that of *S. pombe* chromosome I DNA, whose length is about 7 megabases (27; J.-B. Fan and C. R. Cantor, unpublished data). Estimates of the sizes of these large *N. crassa* chromosomal DNAs must be considered tentative because no molecular weight markers exist in this size range. However, it is likely that these DNAs are longer than 9 megabases. These estimates are based on the migrations of the smaller *N. crassa* chromosomal DNAs relative to those of the *S. pombe* DNAs, combined with the relative sizes of aceto-orcein-stained pachytene chromosomes (12). LG V and LG I are 1.3 and 1.8 times the size of LG IV, respectively (12). LG IV, the third largest chromosomal DNA, is slightly longer than 7 megabases based on its mobility relative to that of chromosome I DNA of *S. pombe*. These values suggest that LG V DNA is longer than 9.1 megabases and LG I DNA is longer than 12.6 megabases. The 9.1-megabase size for LG V is likely to be an underestimate. The DNA of the nucleolus organizer is not condensed in pachytene chromosomes and was not considered when comparing the relative size of this chromosome with those of the others. The nucleolus organizer contains 200 copies of the 9.2-kilobase rDNA repeat unit (17), or 1.8 megabases of DNA. Thus, LG V is probably over 10.9 megabases long.

DNA molecules in the range of 10 megabases, such as those for LG I and LG V, have contour lengths of about 3 mm (28). There is some evidence that large DNA molecules become very extended when placed in alternating electric fields (23). If this is the case, it could explain the broad bands obtained with the larger *N. crassa* chromosomal DNAs (Fig. 1 and 2).

Estimates of the sizes of the four smaller chromosomal DNAs suggest that LG III DNA is 6 megabases (based on its intermediate position between *S. pombe* chromosome I and II DNAs, which are 7 and 5 megabases, respectively). LG II DNA appears to be about 4.6 to 5 megabases based on its mobility relative to that of *S. pombe* chromosome II DNA and the sizes of the two minichromosomes in strains *T(AR179)* and *T(ALS176)* that contain the individual arms of LG II and the LG II centromere. The two smallest *N. crassa* chromosomal DNAs, LG VI and VII, are estimated to be about 4 megabases based on their mobilities relative to those of chromosome II and III DNAs of *S. pombe*. These combined estimates indicate that the total genome of *N. crassa* is longer than 47 megabases. This estimate is significantly greater than the 27-megabase estimate of Krumlauf and Marzluf (9) and close to the 45-megabase estimate of Horowitz and Macleod (8). Precise determination of individual chromosomal DNA sizes, and thus the genome size, awaits the development of accurate high-molecular-weight markers or detailed restriction analyses of individual chromosomal DNAs.

Comparisons of the physical sizes of chromosomes with the genetic map lengths of the *N. crassa* linkage groups are limited by the admittedly imperfect map length estimates (15). LG I is the largest and LG VII one of the smallest by both measurements, whereas the other five linkage groups are intermediate, but their genetic map length order does not correspond to their physical size.

Although LG VI and LG VII DNAs from wild-type strain *74A* were not separable under the conditions used in this study, translocation strains allowed separation of these molecules. LG VI DNA of strain *T(VIIL→IVR)T54M50* migrates as a unique band because the migration of LG VII DNA has been altered. Translocation strains also exist in which the size of LG VI DNA is altered without affecting LG VII DNA. By using appropriate strains, therefore, it should be possible to separate all seven *N. crassa* chromosomal DNAs and rapidly map cloned genes to linkage groups by hybridization. In addition, individual chromosomal DNAs or minichromosome molecules could be purified and used to prepare chromosome-specific libraries or as probes of genomic libraries.

Historically, *N. crassa* chromosomes have designated on the basis of length, using arabic numerals in descending order (12). Linkage groups were subsequently assigned roman numerals in chronological order of discovery (1). Electrophoretic separation of the chromosomal DNAs allows a redefinition of the linkage group-chromosome size relationship. Our data show that LG I corresponds to the largest chromosomal DNA, followed in descending order by LG V, IV, III, II, [VI, VII]. This order differs from the cytological estimate reported by Perkins and Barry (15), who predicted that LG III is larger than LG IV and LG VI is larger than LG II. Because of the extensive genetic characterization of the seven *Neurospora* linkage groups (16), we propose that henceforth *N. crassa* chromosomes should be designated by their linkage group number. This will avoid confusion between the old designations of chromosome numbers and our refinements based on molecular size.

Many fungi are thought to have genomes in the same size range as that of the *N. crassa* genome, and therefore the methods described here should be applicable to the separation of their chromosomal DNAs. We have used similar methods to prepare and separate chromosomal DNAs of the plant-pathogenic fungus *Cochliobolus heterostrophus* (C. R. Bronson, M. J. Orbach, and D. Vollrath, unpublished results). For most fungi there is little or no genetic data; thus, a molecular karyotype combined with physical mapping studies should aid in the identification and isolation of genes of interest.

ACKNOWLEDGMENTS

We thank David Perkins for *N. crassa* strains and helpful discussions. We also thank Gilbert Chu for helpful discussions. We are grateful to Ed Barry, Anne Roberts, Tom Schmidhauser, and Matthew Springer for critical comments regarding the manuscript.

This work was supported by grant DMB 8208866 from the National Science Foundation, Public Health Service grants GM 21891 and AG02908 from the National Institutes of Health, and a grant from the MacArthur Foundation. D.V. was supported by the Medical Scientist training program at Stanford University. C.Y. is a Career Investigator of the American Heart Association.

LITERATURE CITED

1. **Barratt, R. W., D. Newmeyer, D. D. Perkins, and L. Garnjobst.** 1954. Map construction in *Neurospora crassa*. Adv. Genet. **6:** 1–93.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
3. **Buxton, F. P., and A. Radford.** 1983. Cloning of the structural gene for orotidine 5′-phosphate carboxylase of *Neurospora crassa* by expression in *Escherichia coli*. Mol. Gen. Genet. **190:** 403–405.
4. **Carle, G. F., and M. V. Olson.** 1985. An electrophoretic karyotype for yeast. Proc. Natl. Acad. Sci. USA **82:**3756–3760.
5. **Chu, G., D. Vollrath, and R. W. Davis.** 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234:**1582–1585.

6. **Denhardt, D. T.** 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
7. **Feinberg, A., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
8. **Horowitz, N. H., and H. Macleod.** 1960. The DNA content of *Neurospora* nuclei. Microb. Genet. Bull. **17**:6–7.
9. **Krumlauf, R., and G. A. Marzluf.** 1979. Characterization of the sequence complexity and organization of the *Neurospora crassa* genome. Biochemistry **18**:3705–3713.
10. **Legerton, T. L., and C. Yanofsky.** 1985. Cloning and characterization of the multifunctional *his-3* gene of *Neurospora crassa*. Gene **39**:129–140.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **McClintock, B.** 1945. *Neurospora*. I. Preliminary observations of the chromosomes of *Neurospora crassa*. Am. J. Bot. **32**:671–678.
13. **Mortimer, R. K., and D. Schild.** 1985. Genetic map of *Saccharomyces cerevisiae*, edition 9. Microbiol. Rev. **49**:181–213.
14. **Orbach, M. J., E. B. Porro, and C. Yanofsky.** 1986. Cloning and characterization of the gene for β-tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. Mol. Cell. Biol. **6**:2452–2461.
15. **Perkins, D. D., and E. G. Barry.** 1977. The cytogenetics of *Neurospora*. Adv. Genet. **19**:133–285.
16. **Perkins, D. D., A. Radford, D. Newmeyer, and M. Björkman.** 1982. Chromosomal loci of *Neurospora crassa*. Microbiol. Rev. **46**:426–570.
17. **Russell, P. J., S. Wagner, K. D. Rodland, R. L. Feinbaum, J. P. Russell, M. S. Bret-Harte, S. J. Free, and R. L. Metzenberg.** 1984. Organization of the ribosomal ribonucleic acid genes in various wild-type strains and wild-collected strains of *Neurospora*. Mol. Gen. Genet. **196**:275–282.
18. **Schechtman, M. G., and C. Yanofsky.** 1983. Structure of the trifunctional *trp-1* gene from *Neurospora crassa* and its aberrant expression in *E. coli*. J. Mol. Appl. Genet. **2**:83–99.
19. **Schwartz, D. C., and C. R. Cantor.** 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell **37**:67–75.
20. **Singleton, J. R.** 1953. Chromosome morphology and the chromosome cycle in the ascus of *Neurospora crassa*. Am. J. Bot. **40**:124–144.
21. **Smith, C. L., T. Matsumoto, O. Niwa, S. Klco, J.-B. Fan, M. Yanagida, and C. R. Cantor.** 1987. An electrophoretic karyotype for *Schizosaccharomyces pombe* by pulsed field gel electrophoresis. Nucleic Acids Res. **15**:4481–4489.
22. **Snell, R. G., and R. J. Wilkins.** 1986. Separation of chromosomal DNA molecules from *C. albicans* by pulsed field gel electrophoresis. Nucleic Acids Res. **14**:4401–4406.
23. **Stellwagen, N. C.** 1985. Orientation of DNA molecules in agarose gels by pulsed electric fields. J. Biomol. Struct. Dyn. **3**:299–314.
24. **Van der Ploeg, L. H. T., D. C. Schwartz, C. R. Cantor, and P. Borst.** 1984. Antigenic variation in *Trypanosoma brucei* analyzed by electrophoretic separation of chromosome-sized DNA molecules. Cell **37**:77–84.
25. **Vogel, H. J.** 1964. Distribution of lysine pathways among fungi: evolutionary implications. Am. Nat. **98**:435–446.
26. **Vollmer, S. J., and C. Yanofsky.** 1986. Efficient cloning of genes of *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **83**:4869–4873.
27. **Vollrath, D., and R. W. Davis.** 1987. Resolution of greater than 5 mega-basepair DNA molecules by contour-clamped homogeneous electric fields. Nucleic Acids Res. **15**:7865–7876.
28. **Watson, J. D., and F. H. C. Crick.** 1953. A structure for deoxyribose nucleic acid. Nature (London) **171**:737–738.
29. **Yanisch-Perron, L., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13 mp18 and pUC19 vectors. Gene **33**:103–119.