Electrophoretic karyotype of Aspergillus nidulans

(chromosome separation/karyotype/pulsed-field gel electrophoresis/filamentous fungi)

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ABSTRACT An electrophoretic karyotype of Aspergillus nidulans has been obtained using contour-clamped homogeneous electric field gel electrophoresis. Six chromosomal bands were separated, with two of the bands migrating as doublets. Using the Schizosaccharomyces pombe and Saccharomyces cerevisiae chromosomes as size standards, we estimate the sizes of the chromosomes to be between 2.9 and 5.0 megabase pairs (Mb) with a total genome size of approximately 31 Mb. Four of the eight genetic linkage groups were assigned to chromosomal bands by hybridization of contour-clamped homogeneous electric field gel blots with various radiolabeled probes each specific to a particular linkage group. Contour-clamped homogeneous electric field gel analysis of reciprocal translocation strains gave chromosomal assignments for the four remaining linkage groups. In order of decreasing size, the A. nidulans chromosomes are: VIII (5.0 Mb), VII (4.5 Mb), II (4.2 Mb), I and V (3.8 Mb), III and VI (3.5 Mb), and IV (2.9 Mb).

The development of pulsed-field gel electrophoresis technology has extended the size range for separation of linear double-stranded DNA molecules to about 10 megabase pairs (Mb) (1). This technique along with a refinement known as contour-clamped electric field (CHEF) gel electrophoresis (2) has been applied to resolve the genomes of several organisms into defined chromosomal bands (2–5). This has led to the development of electrophoretic karyotypes for Schizosaccharomyces (Sc.) pombe (6, 7), Saccharomyces (Sa.) cerevisiae (8), and Neurospora crassa (1) with genome sizes estimated at 14 Mb, 15 Mb, and 47 Mb, respectively (1, 9, 10).

Aspergillus nidulans is a filamentous ascomycete possessing a haploid complement of eight genetic linkage groups (LGs) (11), with an estimated genome size of 26 Mb (12). We have used the CHEF gel technique to resolve the A. nidulans genome into six bands of chromosomal DNA. Four of these bands contain a single chromosomal DNA, whereas the others each contain two chromosomal DNAs. A comparison of the A. nidulans chromosomes to those of Sc. pombe and Sa. cerevisiae suggests an estimated genome size of 31 Mb for A. nidulans. A combination of Southern analysis using LG-specific probes and CHEF analysis of chromosomes from strains bearing reciprocal chromosome translocations was used to assign each of the chromosomal bands to a specific LG.

MATERIALS AND METHODS

Strains and Hybridization Probes. Intact chromosomal DNA molecules were prepared from the following A. nidulans strains (obtained from the Fungal Genetics Stock Center, Kansas City, KS): Glasgow wild-type FGSC4; FGSC27 T1(V1;VII); FGSC40 T1(V;VI); FGSC250 T1(IV;VIII); FGSC429 T1(II;VII); and FGSC395 T1(III;VII). Sa. cerevisiae strain 334 (Beckman) and Sc. pombe wild-type strain 972 (h−) (13) were used as sources of chromosomal DNA for size markers.

The following cosmids containing cloned genes from A. nidulans were used as radiolabeled probes: cosLys12 (lysE, LG I) (W. Timberlake, personal communication; B. Fishel, personal communication). L7C3 (wetA, LG VII) (14). Plasmids used as radiolabeled probes are as follows: pSal/argB contains a 1.9-kilobase Sal I DNA fragment including the A. nidulans argB gene (15) (LG II) inserted into the Sal I site of pUC4; plasmid pHY201 contains the trpC gene of LG VIII on a 4.1-kilobase Xho I fragment cloned into the Sal I site of pBR329 (16).

Preparation of Intact Chromosomal DNAs. Agarose plugs containing Sc. pombe or Sa. cerevisiae intact chromosome DNAs were either purchased from Beckman or prepared as described (6). Intact Aspergillus chromosomal DNA was prepared as follows: One liter of YG medium (17) was inoculated with 1–3 × 106 conidia and incubated at 32°C for 18 hr with vigorous shaking. Mycelial cells were harvested by filtration through Miracloth (Calbiochem), rinsed briefly with glass-distilled water, and gently squeezed to remove excess liquid. The cells (10–30 g) were suspended in 30 ml of OM buffer (1.2 M MgSO4/10 mM sodium phosphate, pH 5.8) (15), and a solution of 300 mg of Novozyme 234 (Novo Industries, Danbury, CT) and 150 mg of bovine serum albumin in 40 ml of OM buffer was added. The cells were made into protoplasts by incubation for 1–2 hr at 32°C with gentle swirling. The suspension was filtered through Miracloth and 12 ml of filtrate containing the protoplasts were transferred to centrifuge tubes and overlaid carefully with 10 ml of ST buffer (0.6 M sorbitol/10 mM Tris-HCl, pH 7.0) (16). The tubes were centrifuged at 5000 rpm in a HB-4 swinging bucket rotor (Sorvall) for 15 min at 4°C. The banded protoplasts were removed using a bent Pasteur pipet and mixed with an equal volume of STC buffer (1.2 M sorbitol/10 mM Tris-HCl, pH 7.5/10 mM CaCl2) (16). The protoplasts were then pelleted at 7000 rpm in a HB-4 swinging bucket rotor (Sorvall) for 10 min at 4°C and washed twice with 10 ml of STC buffer. The pellet was resuspended in GMB buffer (0.125 M EDTA, pH 7.5/0.9 M sorbitol) such that the concentration of protoplasts was 2–2.5 × 107 cells per ml. The suspension was then placed at 37°C, an equal volume of molten 1.4% InCert agarose (FMC) in GMB buffer precooled to 42°C was added, and the agarose/protoplast mixture was poured into a plug mold (2 × 2 × 25 mm) and solidified on ice for 10 min. The agarose plugs were immersed in NDS buffer (0.5 M EDTA, pH 8.0/10 mM Tris-HCl, pH 9.5/1% sodium N-lauroylsarcosinate) (6) containing proteinase K (2 mg/ml) at 50°C for 24 hr. Finally, the plugs were washed two or three times in 50 mM EDTA (pH 8.0) at 50°C and stored at 4°C in EDTA. The chromosomal DNA in the plugs remains intact for at least 4 months.

CHEF Gel Conditions. CHEF analysis was performed using an apparatus described by Chu et al. (2) except that

Abbreviations: CHEF, contour-clamped homogeneous electric field; Mb, megabase pairs; LG, linkage group; Sa., Saccharomyces; Sc., Schizosaccharomyces.
vertical rather than horizontal electrodes were employed. A 40-ml gel containing 0.8% NDS agarose (National Diagnostics, Highland Park, NJ) was poured directly into a mold (8 × 8 × 1 cm) in the apparatus. The DNA-agarose plugs were inserted into the gel wells and sealed with 0.8% SeaPlaque agarose (FMC). Gels were electrophoresed at 12°C in 0.5× TAE buffer (18) at 45 V with three pulse intervals of 50 min, 45 min, and 37 min at durations of 72 hr, 12 hr, and 72 hr, respectively. The gels were stained in ethidium bromide (0.5 μg/ml) for 45 min and then destained in water for 1 hr.

Hybridization Conditions. CHEF gels were soaked successively at room temperature in 0.25 M HCl (two 15-min periods), in 0.5 M NaOH/1.0 M NaCl (two 10-min periods), then neutralized in 0.5 M Tris-HCl, pH 7.5/1.5 M NaCl (two 10-min periods). The DNA was transferred to a Nytran membrane (Schleicher & Schuell) for 18 hr in 5× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). Cosmid or plasmid DNA (100–300 ng) was labeled to a specific activity of >8 × 10⁶ cpm/μg by using the random-hexamer-priming method (Boehringer Mannheim) and [α-³²P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq) (Amersham). Hybridizations were carried out for 18 hr at 65°C in 0.5 M NaCl/0.1 M sodium phosphate, pH 7.0/6 mM EDTA, pH 8.0/1% SDS/denatured salmon sperm DNA (100 μg/ml) using a probe concentration of 1–2 × 10⁶ cpm/ml. The blots were then washed at 57°C twice for 20 min in 2× SSC/1% SDS and twice for 20 min in 0.5× SSC and subjected to autoradiography.

RESULTS

Intact chromosomal DNA from wild-type A. nidulans strain FGSC4 was released from mycelial protoplasts in agarose plugs and subjected to CHEF electrophoresis. When consecutive switching intervals of 50 min, 45 min, and 37 min were used for a total of 156 hr, the Aspergillus genome was resolved into six bands (Fig. 1, lane c). The relative intensities of ultraviolet fluorescence of the bands after ethidium bromide staining suggested that two of the bands were doublets, indicating the chromosome number in Aspergillus to be eight. This is in agreement with the genetic and cytological determination of eight LGs for this organism (11, 19).

The three Sc. pombe chromosomes are estimated to have sizes of 3.5 mb, 4.7 mb, and 5.5 mb (10), whereas the largest Sa. cerevisiae chromosome (XII) is estimated to be 3.1 mb (20). Using the CHEF-resolved chromosomes from Sc. pombe and Sa. cerevisiae as size markers (Fig. 1, lanes a and b), we estimate the sizes of the Aspergillus chromosomes, in descending order, to be 5.0 mb, 4.5 mb, 4.2 mb, a doublet at 3.8 mb, a doublet at 3.5 mb, and 2.9 mb. The total Aspergillus genome is therefore approximately 31 mb. This value agrees reasonably well with the 26-mb haploid genome size estimated by DNA-DNA reassociation analysis (12).

The electrophoretic gel bands were assigned to specific LGs by a combination of two methods: (i) hybridization with labeled DNA probes containing genes that are genetically mapped and (ii) examination of the CHEF gel band patterns of genomic DNAs from various known chromosomal localization strains. We obtained cosmid and plasmid DNA clones bearing genes specific to four genetic LGs. Cosmids coslysl2 (lysF, LG I) and L7C3 (wetA, LG VII), and plasmids pHY201 (trpC, LG VIII) and pSal/argB (argB, LG III) were radiolabeled and used to probe CHEF gels of resolved A. nidulans chromosomes (Fig. 2). The LG VIII-specific trpC probe was found to hybridize to the 5.0-mb band (Fig. 2, lane b), and the LG VII-specific probe L7C3 hybridized to the 4.5-mb band (Fig. 2, lane c). The LG I-specific probe coslysl2 hybridized predominantly to the 3.8-mb doublet (Fig. 2, lane d), although a weak homology was observed with the 5.0-mb band assigned to LG VIII due to the presence within the cosmid vector pKBY2 of a 4.1-kilobase Xho I fragment containing the trpC gene (21). The LG III-specific probe pSal/argB containing the argB gene hybridized to the 3.5-mb doublet (Fig. 2, lane e). Thus the 5.0-mb, 4.5-mb, 3.8-mb, and 3.5-mb bands correspond to LGs VIII, VII, I, and III, respectively.

![Fig. 1](image1.png)

**Fig. 1.** Separation of A. nidulans intact chromosomal DNAs on a CHEF gel. Chromosomal DNAs were prepared from Sc. pombe strain 972 (lane a), Sa. cerevisiae strain 334 (lane b), and A. nidulans wild-type strain FGSC4 (lane c). The approximate lengths of the resolved chromosome bands are indicated.

![Fig. 2](image2.png)

**Fig. 2.** Hybridization analysis of CHEF-resolved A. nidulans chromosome DNAs. Agarose plugs containing intact chromosomal DNAs from A. nidulans wild-type strain FGSC4 (lanes a–e) were electrophoresed and stained with ethidium bromide, and the DNAs were transferred to a nylon membrane. Lanes: a, ethidium bromide stain; b–e, hybridization of separated chromosomes with labeled DNA probes specific to four of the eight A. nidulans LGs. After transfer of the DNAs to the nylon membrane, the membrane was cut and probed. The genes used as probes are indicated above the autoradiogram. The LGs of the probes are as follows: trpC, LG VIII; wetA, LG VII; lysF, LG I; and argB, LG III.
LG assignment of the remaining gel bands was accomplished by CHEF analysis of reciprocal translocation strains. Chromosomal DNAs were prepared from *Aspergillus* strains bearing reciprocal translocations occurring between chromosomes assigned by hybridization analysis and those LGs not yet assigned to a chromosomal gel band. It was expected that in some strains the translocation would result in observable shifts in gel migration of the two affected chromosomes, allowing the assignment of a LG to a previously unidentified gel band. For example, *A. nidulans* strain FGSC429 possesses a reciprocal translocation between LG II and LG VII (Fig. 3, lane d). CHEF analysis of chromosomal DNAs from this strain revealed shifts in gel migration for the identified 4.5-mb band assigned to LG VII and the 4.2-mb gel band. This result assigns the 4.2-mb gel band to LG II and confirms the assignment of LG VII.

To assign LG IV, we analyzed the DNA from reciprocal translocation strain FGSC250 T1(IV; VIII). The translocation breakpoints in this strain occur on the right arm of LG IV close to the centromere and on the outer right arm of LG VIII (22). As a result of the translocation, it was expected that the relative sizes of LG IV and LG VIII would change significantly. CHEF electrophoresis of DNA from FGSC250 showed gel migration shifts for both the 5.0-mb gel band previously assigned to LG VIII by hybridization analysis and the 2.9-mb gel band (Fig. 3, lane b). The 2.9-mb gel band was therefore assigned to LG IV.

CHEF gel electrophoresis of chromosomal DNAs from strain FGSC27 T1(VI; VIII) revealed shifts in gel migration for the 4.5-mb LG VII band as well as for one of the bands comprising the 3.5-mb doublet (Fig. 3, lane f). It can be concluded that in addition to LG III, the 3.5-mb doublet contains the chromosome corresponding to LG VI. Southern analysis performed on CHEF-resolved DNA from strain FGSC27 using radiolabeled pSal/argB DNA confirmed that the single 3.5-mb chromosome corresponds to LG III (data not shown).

Analysis of the chromosomal bands of FGSC40 T1(V; VI) revealed shifts in single components of the 3.5-mb doublet (LG VI) and the 3.8-mb doublet (Fig. 3, lane e), identifying one component of the latter band as LG V. Southern analysis of CHEF-resolved FGSC40 DNA using radiolabeled cosyl12 was performed to confirm that the remaining gel band of the 3.8-mb doublet corresponds to LG I (data not shown).

The CHEF analysis of DNA from the translocation strain FGSC429 T1(II; VII) (Fig. 3, lane d) revealed a minichromosome exhibiting gel mobility equivalent to that of *Sa. pombe* chromosome IV, which has an approximate size of 1.6 mb (9, 20). Since the sizes of wild-type LG II and LG VII were measured as 4.2 mb and 4.5 mb, respectively, the larger translocation product should measure approximately 7.1 mb. Because of the unusually wide range of chromosomal sizes, intact DNAs from translocation strain FGSC429 can be quite useful size markers when resolving chromosomal bands between 1.6 and 7.0 mb.

**DISCUSSION**

Based on the approximate sizes previously assigned to the *Sc. pombe* and *Sa. cerevisiae* chromosomes (6, 9, 10), we estimate the sizes of the *A. nidulans* chromosomes to be 5.0 mb, 4.5 mb, 4.2 mb, a doublet of 3.8 mb, a doublet of 3.5 mb, and 2.9 mb. These results agree with early cytological experiments that proposed that the *Aspergillus* genome contains eight chromosomes grouped into three size classes, with three large chromosomes, four chromosomes of similar intermediate size, and one small chromosome (19). The estimated total genome size based on CHEF analysis is 31 mb, which agrees with the 26 mb estimate of Timberlake (12), obtained using DNA-DNA reassociation analysis. Chromosome-specific radiolabeled probes were used to identify four of the *Aspergillus* chromosome bands resolved by CHEF gel electrophoresis. Hybridization analysis using a hybridization probe containing lysF assigned one of the 3.8-mb doublet bands to LG I. This assignment was confirmed using as hybridization probe cosmid pKBY3, which contains the LG I-specific pabaA gene (W. Timberlake, personal communication) (data not shown). In many cases, we could verify the LG assignments based on hybridization data by examining the band patterns exhibited by chromosomal translocation strains. For example, assignment of LG VIII to the largest (5.0 mb) gel band by hybridization to a trpC probe was verified by CHEF electrophoresis of DNA from translocation strain FGSC250 T1(IV; VIII) (Fig. 3, lane b). Translocation strains FGSC27 T1(VI; VII) (Fig. 3, lane f) and FGSC429 T1(II; VIII) (Fig. 3, lane d) each exhibit altered mobility for the 4.5-mb gel band assigned to LG VII by hybridization with a wetA probe. Confirmation of LG III, first assigned to the 3.5-mb band by hybridization to an argB probe, employed CHEF analysis of FGSC395 T1(III; VII). In DNA from that translocation strain, the 4.5-mb band displayed a decrease in mobility of approximately 0.1 mb with a corresponding broadening of the 3.5-mb doublet (Fig. 3, lane c).

The physical sizes of the chromosomes were compared with the genetic lengths of the eight *Aspergillus* LGs. Although this comparison is limited due to the imperfect map lengths (23), there are consistencies between the cytological and electrophoretic analyses. The CHEF and genetic linkage data agree that LGs VII, VIII, and II are the largest, with LG I, V, VI, and III of intermediate size, and establish LG IV as the smallest.

In the past the chromosomes of *Aspergillus* were identified by either cytological or genetic analysis. Cytological identi-
Fractionation designated the chromosomes on the basis of decreasing length using arabic numerals (19), whereas genetic LGs were assigned Roman numerals in chronological order of their discovery (11). The development of an electrophoretic karyotype of *Aspergillus* now permits correlation of the cytological and genetic linkage data by making direct comparisons between physical sizes of the chromosomes and their genetic makeup. Thus, chromosomes 1 to 8 as determined electrophoretically correspond in order of decreasing size to LG VIII; LG VII; LG II; [LG I and LG V]; [LG III and LG VI]; and LG IV.

Often DNA sequences from *Aspergillus* are isolated from genomic libraries by selecting for complementation of auxotrophic mutations in either homologous or heterologous systems. Hybridization analysis using cloned DNA sequences as probes to the electrophoretic karyotype can be used in conjunction with genetic analysis to determine the chromosomal origin and genetic location of these sequences. In addition, the use of CHEF fractionation of *Aspergillus* chromosomes can provide a source of DNA for the construction of chromosome-specific DNA libraries.

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