Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 3756–3760, June 1985
Genetics

An electrophoretic karyotype for yeast

(DNA/chromosomes/orthogonal-field-alternation gel electrophoresis)

GEORGES F. CARLE AND MAYNARD V. OLSON

Department of Genetics, Box 8031, Washington University School of Medicine, St. Louis, MO 63110

Communicated by Herschel L. Roman, January 28, 1985

ABSTRACT **The chromosomal DNA molecules of a standard laboratory strain of *Saccharomyces cerevisiae* have been separated into 12 well-resolved bands by orthogonal-field-alternation gel electrophoresis. DNA·DNA hybridization probes derived from cloned genes have been used to correlate this banding pattern with yeast's genetically defined chromosomes. The 12 bands are shown to represent 9 singlets and 3 comigrating doublets, thereby accounting for 15 chromosomes that were identified as I–XI and XIII–XVI. Because the three comigrating doublets could be readily resolved in certain laboratory yeast strains that contain chromosome-length polymorphisms relative to our standard strain, all 15 of these chromosomes could be displayed as a single band in at least one of four strains that were studied. A 16th chromosome (number XII), which is known to contain the genes for rRNA, does not reproducibly enter the gels. By making use of the band identifications, the previously unmapped fragment F8 was assigned to chromosome XIII. With the possible exception of chromosomes that differ greatly in size or electrophoretic behavior from all the known chromosomes, the results appear to define a complete "electrophoretic karyotype" for yeast.**

The advent of electrophoretic techniques for separating the intact chromosomal DNA molecules of lower eukaryotes (1–5), has provided a novel means of characterizing the chromosome sets of these organisms. This technique may be expected to provide fundamentally new information about the basic organization of the genomes of many species, since numerous members of such taxonomic groups as the protozoans and fungi have proven intractable both to genetic and cytogenetic analysis. Conversely, only in rare cases—such as *Neurospora* (6)—has there been a productive marriage between the genetic definition of linkage groups and the cytogenetic characterization of chromosomes as physical entities.

In the case of the yeast *Saccharomyces cerevisiae*, the ability to enumerate and distinguish between the chromosomes has rested almost entirely on linkage analysis. Genetic mapping has suggested that the yeast genome is partitioned amongst approximately 17 chromosomes (7), a result that implies an average chromosome size of 500–1000 kilobase pairs (kb). The small sizes and poorly defined mitotic and meiotic morphologies of the yeast chromosomes have precluded the development of a useful karyotype by light microscopy. Electron microscopy, particularly as applied to the tracing of synaptonemal complexes, has been more successful, but even this technique has failed to provide a precise chromosome count or to define the relative sizes of the genetically defined chromosomes (8).

The idea of using the electrophoretic fractionation of intact chromosomal DNA molecules as an alternative to classical karyotyping has been explored for many years (9). However, efforts to apply electrophoresis to the separation of very large DNA molecules achieved only moderate success until 1982, when it was reported by Schwartz *et al.* (1) that molecules of several hundred kilobase pairs had strongly size-dependent mobilities when they migrated through agarose gels in the presence of two alternately applied, approximately orthogonal electrical fields. More recently, Schwartz and Cantor introduced a technique for releasing DNA from yeast spheroplasts that had been embedded in agarose, thereby preserving the intactness of the yeast chromosomal DNA molecules, and they also showed that several single-copy DNA·DNA hybridization probes hybridized to specific bands that could be separated by the orthogonal-field-alternation technique (2). We have independently reported similar hybridization experiments, and we have developed an electrophoresis apparatus on which DNA molecules over the entire size range of the yeast chromosomes can be separated on a single gel into a series of easily visualized bands (4).

We have now combined our implementation of the orthogonal-field-alternation gel electrophoresis (OFAGE) technique with an embedded-agarose method of sample preparation to carry out a comprehensive analysis of the yeast chromosome set. This analysis made use of DNA·DNA hybridization probes for 16 of the 17 chromosomes that have been proposed on the basis of genetic data. In our standard yeast strain, the chromosomal DNA molecules separate into 12 well-resolved bands, which appear—both from their intensities and from the hybridization data—to comprise 9 singlets and 3 doublets. All three of the doublets are readily resolved in certain yeast strains, which contain chromosome-length polymorphisms (CLPs) relative to our standard strain. One chromosome, number XII, does not reproducibly enter the gels under our experimental conditions. Consequently, by using a set of four yeast strains, we were able to resolve all 16 of the chromosomes that we analyzed.

MATERIALS AND METHODS

Electrophoretic Protocol. The apparatus in which we carried out the OFAGE has been described in detail (4). Our standard electrophoretic conditions (e.g., Figs. 1 and 2) employed 1.5% agarose, 300 V, 13°C, a running time of 18 hr, and a switching interval of 50 sec. Minor variations from these conditions are noted in the captions to Figs. 3 and 4. Other basic experimental procedures, such as the techniques for staining and photographing the gels, as well as analyzing them by DNA·DNA hybridization, have also been described (4). The only modification of our previously published techniques for staining the gels is that we now destain the gels in water for long periods (1–2 days) when maximal contrast between the bands and the background is desired; no restaining is necessary.

Sample Preparation. We prepared the samples of yeast DNA by a modification of the embedded-agarose procedure

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Abbreviations: kb, kilobase pair(s); OFAGE, orthogonal-field-alternation gel electrophoresis; CLP, chromosome-length polymorphism; cM, centimorgan(s); rDNA, DNA encoding rRNA.

of Schwartz and Cantor (2). Specifically, samples are prepared as follows: Cells are grown as previously described (10) to late logarithmic phase in 100 ml of a rich, glucose-based liquid medium and then harvested and washed twice with 50 mM EDTA, pH 7.5 at 0°C; the final cell pellet is suspended in 3.25 ml of 50 mM EDTA, pH 7.5; 3 ml of the cell suspension is mixed at 37°C with 5 ml of 1% low-gelling-temperature agarose (prepared in 0.125 M EDTA, pH 7.5, and cooled to 42°C) and 1 ml of solution I [prepared by mixing 10 ml of SCE buffer (10), 0.5 ml of 2-mercaptoethanol, and 10 mg of zymolyase 60,000 (Miles)]; the mixture of agarose, cells, and cell-wall-removing enzyme is poured into a small Petri plate (diameter 6 cm), allowed to gel at room temperature, overlaid with 5 ml of solution II [0.45 M EDTA, pH 9/10 mM Tris·HCl, pH 8/7.5% (vol/vol) 2-mercaptoethanol], and incubated overnight at 37°C in a sealed plastic bag; the overlay is replaced with 5 ml of solution III [0.45 M EDTA, pH 9/10 mM Tris·HCl, pH 8/1% sodium *N*-lauroylsarcosinate/1 mg of proteinase K (Boehringer Mannheim) per ml], the plastic bag is resealed, and the plate is incubated overnight at 50°C; finally, the overlay is replaced with 0.5 M EDTA, pH 9, and the plate is stored at 4°C. Gels are loaded by cutting a slice of the chilled agarose that will fit into a preparative well formed by a comb with a thickness of 1.7 mm and gently teasing the slice into the well. When multiple strains are loaded onto the same gel, the plugs are simply juxtaposed in a single preparative well. One Petri plate provides enough samples for many gels; in most cases, the best results are obtained if the samples are used within 2 weeks of preparation.

Hybridization Probes. The identities of the DNA·DNA hybridization probes were as follows: chromosome I, *CDC19*, λPM4237 (4); II, *LYS2*, YIp333 (11); III, *SUP61*, λPM680 (unpublished); IV, *SUP2*, λPM1405 (unpublished); V, *URA3*, λPM910 (4); VI, *SUP11*, λPM4235 (4); VII, *LEU1*, λ2-14-4 (J. Margolskee and I. Herskowitz, personal communication); VIII, *ARG4*, pGT30 (12); IX, *SUP17*, 14g (13, 14); X, *URA2*, pJLS1 (15); XI, *URA1*, pRG4 (16); XII, rDNA, λPM4142 (unpublished) and *GAL2*, pTLG2 (J. Tschopp and R. Schekman, personal communication; the 3.9-kb *Bam*HI fragment was gel-purified); XIII, *SUP8*, λPM1003 (unpublished); XIV, *CEN14*, pPM408 [contains a 1.4-kb *Eco*RI/*Hin*dIII fragment subcloned for this work in pBR322 from the plasmid pSC807-A (S. Cwirla, R. Elder, and R. Easton Esposito, personal communication)]; XV, *SUP3*, λPM1420 (unpublished); XVI, *GAL4*, pG525 (17); F8 = XIII, *SUP5*, pBS500 [contains a 5.2-kb *Hin*dIII fragment in pBR322 (unpublished)] and *GAL80*, pBM320 [contains a 3.1-kb *Hin*dIII fragment in pBR322 (R. R. Yocum and M. Johnston, personal communication)]; mitochondrial DNA, p2E [contains a 3.5-kb ω^- *Hpa* II fragment of the DNA for rRNA (rDNA) in pBR322 (A. Hudson, P. Zassenhaus, and R. Butow, personal communication)]; and 2-μm DNA, pSI4 (ref. 18; the 2.2-kb *Eco*RI fragment was gel purified).

RESULTS

The OFAGE Banding Pattern of Total Yeast DNA. Fig. 1 shows the banding pattern that we observe when the chromosomal DNA molecules of our standard yeast strain [the ρ^0 haploid AB972 (19)] are separated under the electrophoretic conditions that we normally employ and visualized by ethidium bromide staining. There are 12 bands, which we number 1–12, in order of increasing size. The pattern is identical to that which we have reported previously (4) with three exceptions, all of which relate to the use of an embedded-agarose method of sample preparation rather than the more conventional DNA-preparation protocol that we employed earlier. First, there is no significant background of partially degraded molecules, while the smaller bands in our previous study were superimposed on an intense smear in the 100- to 500-kb region. Second, with the exception of bands 5, 10, and 11—which the analysis described below indicates are unresolved doublets—the intensities of the bands increase with apparent size in an orderly way, a result that is consistent with the expectation that the molecules are present in equimolar amounts. Finally, band 12 was not detectable in our previous preparations, presumably because it corresponds to a molecule that is too large to survive conventional sample-handling methods.

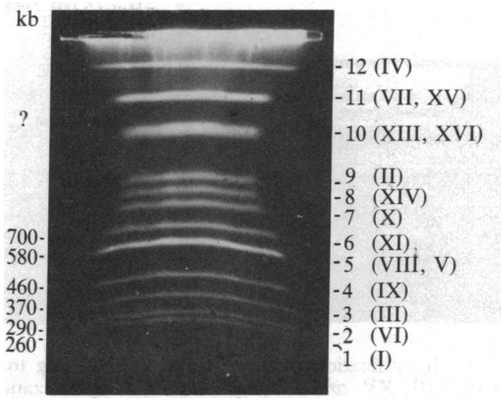

FIG. 1. Ethidium bromide stained agarose gel on which the chromosomal DNA molecules of yeast (strain AB972) have been resolved. The size estimates on the left and the band-numbering system on the right are from ref. 4, while the chromosome assignments summarize the conclusions of the present study.

The size estimates in Fig. 1 are based on the comparison of the mobilities of the smaller yeast chromosomal DNA molecules with those of a variety of bacteriophage DNAs (4), while the chromosome assignments anticipate results that are described below. With respect to the size estimates, it should be noted that no independent size estimates are available for specific yeast chromosomes; consequently, the OFAGE values reflect the untested assumption that the yeast chromosomal DNAs obey the same size–mobility relationship as do the bacteriophage DNAs that were employed as size standards. Although the present data do not address this point directly, the orderly inverse correlation between intensity and mobility that is evident in Fig. 1 at least supports the supposition that the predominant determinant of the mobility of a chromosomal DNA molecule on these gels is its size.

Identification of the Chromosomes that Correspond to Specific OFAGE Bands. Because there is an exceptionally well developed genetic map for yeast and there are powerful methods for cloning genes by functional complementation, large numbers of genes that correspond to mapped genetic loci have been cloned (20, 21). From these cloned genes, we were able to assemble a set of DNA·DNA hybridization probes specific for chromosomes I–XVI, as well as for the mitochondrial genome (22) and the autonomously replicating 2-μm circle (23); only in the case of the recently proposed chromosome XVII, which is defined by a single locus (24), was no DNA·DNA hybridization probe available.

We have used this set of hybridization probes to expand our earlier assignments (4) of chromosomes I (band 1), V (band 5), and VI (band 2), to include chromosomes I–XVI (see the labeling of the bands in Fig. 1). As shown in Fig. 2, which illustrates the results of typical experiments, we obtain strong and specific hybridization signals even from bands corresponding to the largest chromosomes. The absence of significant smearing of the hybridization below the hybridizing band corroborates the impression from the ethidium bromide staining pattern that the molecules are largely intact.

The only chromosome that gave anomalous results was chromosome XII. This chromosome is known to contain all—

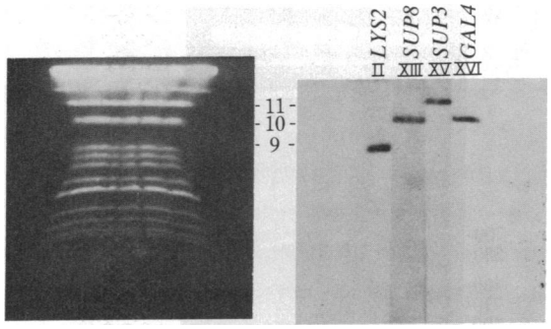

FIG. 2. Identification of the bands corresponding to chromosomes II, XIII, XV, and XVI by DNA·DNA hybridization (strain AB972). DNA from the gel whose ethidium bromide staining pattern is shown on the left was transferred to a single sheet of nitrocellulose. This sheet was then cut into four strips that were separately hybridized to four chromosome-specific probes, and the filter strips were then positioned in their original alignment before the autoradiogram on the right was exposed. The ethidium bromide staining pattern and the autoradiogram are printed to the same scale and are aligned appropriately with one another.

or nearly all—of the genes for rRNA, which are thought to be organized into a single block of over 100 tandem copies of a 9-kb repeating unit (25). We obtained similar results probing for chromosome XII either with a bacteriophage λ clone containing the rRNA genes or with a restriction fragment containing the *GAL2* gene, a single-copy gene that maps to chromosome XII. The results with both probes indicated that chromosome XII behaves both anomalously and irreproducibly under our experimental conditions. The results were anomalous in the sense that chromosome XII never formed a well-defined band that was detectable by ethidium bromide staining, while they were irreproducible in the sense that the distribution of chromosome XII sequences, as judged by hybridization, varied considerably from experiment to experiment. At one extreme, which was the most commonly observed case, no hybridization to chromosome XII sequences was observable except in the immediate vicinity of the well; at the other extreme, hybridization was observed immediately below the well and also in a broad smear that terminated abruptly at a front in the general region of bands 10–12. In the latter cases, the extent to which the front resembled a leading band was quite variable. In general, it was difficult to predict the hybridization results from the ethidium bromide staining pattern, although in the most extreme cases, the band-like front and trailing smear could be clearly observed on the stained gels. The band-like front, in these cases, was less intense than the surrounding bands (nos. 10–12) and not in a reproducible position relative to them. Schwartz and Cantor have also reported anomalous behavior for chromosome XII; the single experiment that they describe resembles our exceptional cases of a band-like front with a trailing smear (2).

We also investigated whether or not mitochondrial DNA or DNA from the yeast 2-μm circle contributes to the ethidium bromide staining pattern of yeast strains that contain these molecules. Our standard strain AB972 is an ethidium bromide induced ρ^0 strain that lacks all mitochondrial DNA (26). When its banding pattern was compared to that of the ρ^+ strain from which it was derived, there was no difference in the region of the gel that contains bands 1–12. However, the ρ^+ strain exhibited a broad, poorly defined band between band 1 and the L double-stranded RNA, a 5-kb molecule that is part of the yeast killer system (27) and that is visible at the bottom of some OFAGE gels (4). This broad band, which is absent from AB972, hybridizes to a probe prepared from cloned mitochondrial rDNA. Although other faint bands are detectable with this probe, the diffuse band below band 1 accounts for the overwhelming majority of the hybridization. This result is not the expected behavior for supercoiled circles the size of yeast mitochondrial DNA (75 kb). We have, for example, run supercoiled circles of a 40-kb derivative of bacteriophage λ on a gel identical to that shown in Fig. 1 and these circles—which were isolated from λ-infected *Escherichia coli* cells—migrated as a sharp band between bands 6 and 7. Similarly, supercoiled monomers of pBR322 (4.6 kb), migrated between bands 3 and 4. We have no explanation for the behavior of mitochondrial DNA, but for the present purposes, the relevant point is that it does not interfere with the analysis of the chromosomal bands, even in the case of ρ^+ strains. We simply note that the mobility of the mitochondrial DNA molecules is comparable to that expected for linear 75-kb molecules.

In the case of 2-μm DNA, DNA·DNA hybridization experiments revealed the presence of multiple bands, whose positions relative to those arising from the chromosomal DNA molecules depended on the switching interval. At a switching interval of 50 sec, the strongest hybridization was to band 3; there was also significant hybridization between bands 7 and 8, although no corresponding feature was detectable by ethidium bromide staining. At a switching interval of 30 sec, the major DNA·DNA hybridization was to a position between bands 5 and 6, where a very faint band was also observable by ethidium bromide staining. Further experiments will be required to determine the correspondence between particular forms of 2-μm DNA and specific bands, but the present results indicate that 2-μm DNA does not give rise to ethidium bromide stained bands of sufficient intensity to interfere with the analysis of the banding pattern that arises from the chromosomal DNA molecules.

CLPs. To maximize the number of bands that could be uniquely associated with particular chromosomes, we investigated a variety of laboratory yeast strains for useful CLPs. Fig. 3 illustrates the success of this approach in the case of band 5, which in our standard strain hybridizes to chromosomes V and VIII. In the widely used strain A364a (28), band 5 is resolved into two components; the one with higher mobility (5A) hybridizes with a chromosome VIII probe, while the lower-mobility component (5B) hybridizes with a chromosome V probe. Although the gel in Fig. 3 was run with a switching interval of 30 sec to maximize resolution in the region where band 5 migrates, bands 5A and 5B in A364a are also adequately resolved under our standard running conditions (i.e., a 50-sec switching interval as in Fig. 1). It is significant that, in A364a, bands 5A and 5B are of equal intensity, a result which indicates that band 5 is only a doublet in AB972, as opposed to a band of higher multiplicity.

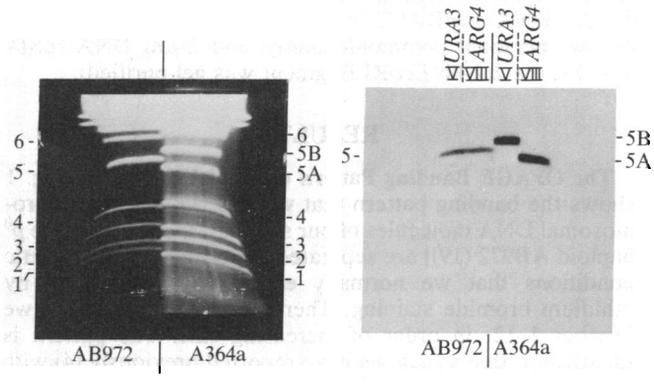

FIG. 3. Resolution of band 5 into two components in strain A364a. The experimental design was analogous to that described in the caption to Fig. 2. The gel was run with a switching interval of 30 sec to optimize resolution in the band 5 region.

We carried out similar experiments on strain YNN281, in which a CLP affecting band 10 was recently discovered (D. Vollrath and R. Davis, personal communication). As shown in Fig. 4*A*, band 10 can be resolved in YNN281 into components 10A (chromosome XIII) and 10B (XVI). A small size difference between chromosomes XIII and XVI is detectable by hybridization even in AB972. The decomposition of band 10 into two easily resolved components in YNN281 arises because of CLPs that affect both chromosomes and accentuate the size difference present in AB972. Once again, the two components of band 10 appear to have equal intensities, suggesting that, in AB972, band 10 is simply a doublet, whose unusual width arises because the two components have significantly different mobilities.

The resolution of band 10 into two components allowed us to map the fragment F8 to chromosome XIII. This fragment, which contains the three linked genes *GAL80*, *SUP5*, and *ARG81*, has remained unassigned to any of the known chromosomes despite several efforts at genetic mapping during a period of more than 10 years (29, 30). We had previously noted that hybridization probes to *GAL80* and *SUP5* hybridized to band 10, and by using YNN281 we were able to refine this assignment to band 10A, which was shown above to correspond to chromosome XIII (see Fig. 4*B*). In independent experiments, D. Schild and R. K. Mortimer (personal communication) have recently mapped *GAL80* and *SUP5* to chromosome XIII with the aid of a genetic mapping technique that is based on the high frequency of mitotic chromosome loss in *rad52/rad52* diploids.

Although CLPs that allow the resolution of bands 5 and 10—particularly band 5—are relatively common amongst laboratory yeast strains, it proved difficult to find CLPs affecting band 11. Nonetheless, we recently discovered that band 11 is resolvable into two components in strain DC04α (ref. 31; Yeast Genetic Stock Center); the components are well separated under the conditions used to run the gel in Fig. 1, and once again, they are of equal intensity. The higher-mobility band, 11A, corresponds to chromosome XV in this strain, while the lower-mobility band, 11B, corresponds to chromosome VII (data not shown). Consequently, all 15 of the chromosomes that enter the gel under our standard experimental conditions can be displayed as a single band in one of the four strains AB972, A364a, YNN281, and DC04α. We have also encountered one case of a single laboratory yeast strain that contains 15 bands, all apparently singlets, and several cases with all the bands except band 11 resolved, but none of these strains has been analyzed in detail.

DISCUSSION

The recently developed technique of OFAGE has been used to carry out a comprehensive analysis of the yeast chromosome set. The analysis was based on separating the chromosomal DNA molecules electrophoretically and identifying the electrophoretic bands by DNA·DNA hybridization, using hybridization probes derived from cloned genetically mapped genes. Probes were available for chromosomes I–XVI, and, in our standard strain, these chromosomes separate into nine singlets and three doublets, while one chromosome fails to enter the gels reproducibly. All three of the doublets were readily resolved in other laboratory yeast strains that contain CLPs relative to our standard strain. The basis of these CLPs amongst interfertile strains is not known, but a likely hypothesis is that the extensive polymorphisms that have recently been described at the ends of the yeast chromosomes are an important component of the phenomenon (32).

Only one other chromosome, XVII, has been proposed on the basis of genetic mapping data, but a hybridization probe is not presently available for the single gene (*KRB1*) that defines this chromosome. Our data raise doubts as to whether chromosome XVII will prove to be a "conventional" chromosome. No bands in the size range of chromosomes I–XVI are unaccounted for in our analysis, and we believe that any smaller chromosomes would also have been detected, if present, down to a lower limit of approximately 20 kb. Furthermore, because we could observe 15 of the chromosomes as apparent singlets, as judged by their intensities on ethidium bromide stained gels, it is unlikely that any chromosomes for which probes were unavailable escaped detection because they migrate with other chromosomes. Consequently, chromosome XVII, or other as yet undiscovered chromosomes, would appear to be constrained to be exceptionally large, exceptionally small, or—as appears to be the case for chromosome XII under our experimental conditions—incapable of reproducibly entering the gels. Given the present stage of development of yeast genetics, only the latter two possibilities are at all probable, since there is little likelihood that a large chromosome would have escaped repeated detection by genetic techniques.

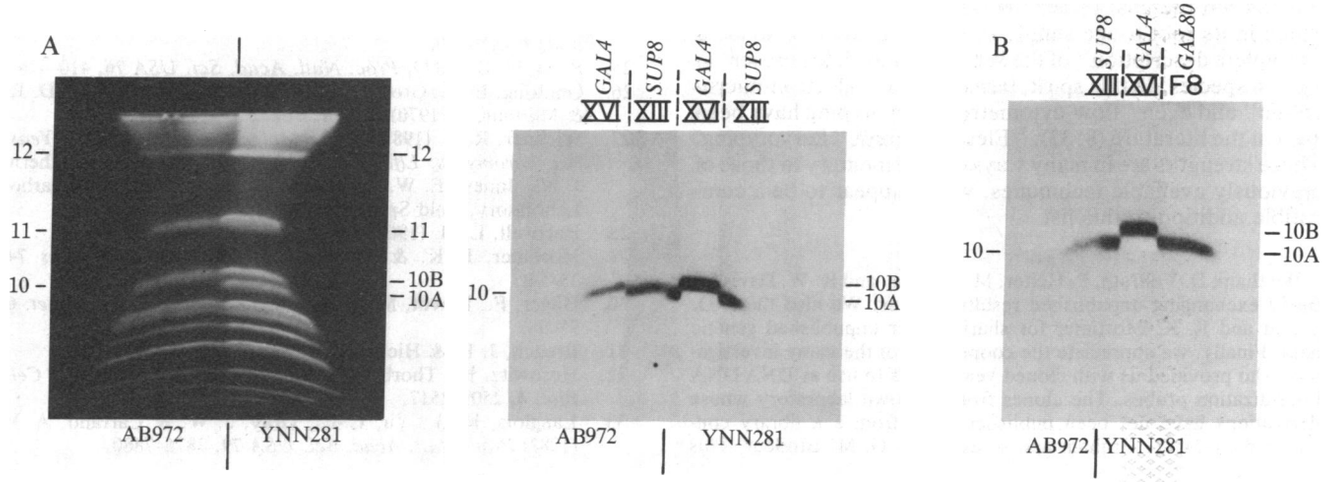

FIG. 4. Resolution of band 10 into two components in strain YNN281 (*A*) and mapping of the F8 fragment to chromosome XIII (*B*). The experimental design was analogous to that described in the legend to Fig. 2. The gel whose ethidium bromide staining pattern is shown was used to prepare the filter shown in *A*; this gel was prepared from 1.2% agarose, which favors separation in the region of bands 10–12. The gel that was used to prepare the filter shown in *B* was run under the same conditions as that in *A* and had a virtually identical appearance. Note that the boundary of filter strips 2 and 3 in *A* and 1 and 2 in *B* do not precisely correspond to the boundaries between the AB972 and YNN281 samples.

We are uncertain as to why chromosome XII normally fails to give rise to a band under our experimental conditions. Our present hypothesis is that the anomalous behavior of chromosome XII arises from its slow and incomplete release from an associated cellular component that is immobilized in the well by the method of sample preparation. An obvious candidate for such a component is the nucleolus, given the presence of the rRNA genes on this chromosome. We cannot, however, rule out the possibility that, amongst the copies of this chromosome, there is extreme heterogeneity in some property that affects its mobility such as the number of copies of the rDNA repeat or the presence of special structures associated with replication or recombination events.

Despite the anomalous behavior of chromosome XII and the failure to detect chromosomes XVII, the data presented here lead to an overall view of the physical structure of the yeast genome that is in striking agreement with the predictions of genetic data. Indeed, it is this agreement that provides the strongest evidence that the bands observed in OFAGE experiments actually correspond to the DNA molecules present in specific yeast chromosomes, since neither the physical underpinnings of the method nor the structures of the resolved molecules have as yet been established by any independent means. Particularly impressive is the generally good correlation between the apparent physical and genetic sizes of the chromosomes. A detailed analysis of this correlation is hampered by the lack of size markers for molecules greater than 700 kb, as well as the existence of many gaps in yeast's meiotic linkage map. Nonetheless, the six chromosomes for which continuous meiotic linkage maps are available are placed in the same size order by recombination distance and OFAGE mobility: chromosome I [90 centimorgans (cM)] = band 1 (260 kb); VI (100 cM) = band 2 (290 kb); III (150 cM) = band 3 (370 kb); V (220 cM) = band 5 (580 kb); II (270 cM) = band 9 (?); IV (430 cM) = band 12 (?)—recombination lengths are from ref. 7, while the OFAGE sizes are from ref. 4. For the four chromosomes for which both physical and genetic size estimates are available, the ratio between the two values varies only from 2.5 to 2.9 kb/cM.

We conclude by commenting briefly on the relationship between "electrophoretic karyotyping" and other methods of characterizing the chromosome sets of eukaryotic organisms. In one sense, this technique differs so greatly from the classical activity of describing the morphologies of chromosomes by light microscopy that it perhaps deserves an altogether different name. Nonetheless, historical usage has emphasized the goal rather than the means of karyotyping, which in its root usage simply refers to efforts to provide a "complete description" of the set of chromosomes present in a given species. In this spirit, terms such as "electron-microscopic" and even "flow-cytometric" karyotyping have been used in the literature (8, 33). "Electrophoretic" karyotyping, whose strengths are in many ways complementary to those of previously available techniques, would appear to be a compatible addition to this list.

We thank D. Vollrath, P. Heiter, M. Thomas, and R. W. Davis for freely exchanging unpublished results with us. We also thank D. Schild and R. K. Mortimer for sharing their unpublished genetic data. Finally, we appreciate the cooperation of the many investigators who provided us with cloned yeast genes to use as DNA·DNA hybridization probes. The clones from our own laboratory whose derivations have not been published were from a λ library constructed by M. Y. Graham and screened by G. M. Brodeur. This work was supported in part by Grant GM28232 from the National Institutes of Health.

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