An electrophoretic karyotype for *Schizosaccharomyces pombe* by pulsed field gel electrophoresis

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ABSTRACT

The three chromosomal DNAs of *S. pombe* have been fractionated by pulsed field gel electrophoresis. The resulting molecular karyotype will greatly speed gene mapping in this organism, and it indicates that the separation range of the technique extends to DNA molecules as large as 9,000,000 base pairs.

INTRODUCTION

Pulsed field gel electrophoresis (PFG) permits convenient and routine separation of very large DNA molecules (1-3). Most or all of the chromosomal DNAs from typical *Saccharomyces cerevisiae* strains can be resolved by PFG (2-5). This provides a very convenient and rapid way of assigning genes to chromosomes and monitoring the entire genomes for any chromosomal rearrangements. The sizes of the *S. cerevisiae* chromosomes can be determined by comparison with linear concatemers of bacteriophage lambda DNA (3, 6). These molecules provide the only set of known DNA sizes in the most common operating range of PFG. The lambda DNAs can range in size from the monomer (48.5 Kb for wild type lambda, 42.5 Kb for lambda vir) up to oligomers as high as the 34-mer (7). These conveniently cover the size range of the *S. cerevisiae* chromosomes.

Quite a few cases have been observed of PFG fractionation of DNA molecules larger than the largest available lambda concatemer. These have been seen in a number of parasitic protozoa including Trypanosomes (8), Leishmania (9), and Plasmodium (10-12). It is possible to make crude estimates of the sizes of these molecules from their relative PFG mobility and the known genome sizes of the organisms. However, in the past such estimates have been quite unreliable because the exact molecular weight dependence of PFG mobility is unknown. For example early estimates of the largest

S. cerevisiae chromosomal DNAs were in excess of 2 Mb (2), while the same molecules compared with lambda DNAs standards, appear to have a molecular weight of only 1.3 Mb (7).

We were interested in trying to extend the operating range of PFG to larger DNA sizes. At the same time, we needed to find a convenient source of DNAs of known size larger than the largest S. cerevisiae chromosomes. The fission yeast Schizosaccharomyces pombe appears to satisfy both of these needs handily. Ordinary haploid strains of S. pombe have three nuclear chromosomes (13-15) in addition to mitochondrial DNA. Additional strains are available with a fourth minichromosome (16). These chromosomes condense in mitosis (17-18), and so they can be visualized clearly in the light microscope. The sizes of these chromosomes can be roughly estimated from their relative staining, the known linkage map, and the known genome size (15). Using these parameters we estimate that the three normal S. pombe chromosomes are about 3 Mb, 6 Mb and 9 Mb. Typical minichromosomes are in the range of 0.3 Mb to 0.5 Mb (16).

MATERIALS AND METHODS

DNA samples. S. pombe strains used were 972, which contains the three normal wild type chromosomes and HM248, which contains, in addition, minichromosome Ch16 (16) and also a cold-sensitive beta tubulin mutation (17,18). Chromosomal DNAs were prepared from these strains as follows (2,16). Late log phase cells were washed in 50 mM EDTA (pH 7.5) and then incubated in 0.3 mg/ml Zymolyase 100T at 37 C for 1-2 hr. in 20 mM citrate-phosphate buffer (pH5.6)-50 mM EDTA-0.9 M Sorbitol. Two ml of spheroblasts were mixed with 2 ml of liquid low gelling agarose (Seaplaque, FMC), distributed in 100 ul molds, and allowed to solidify. The resulting agarose blocks were pushed from the mold into 0.25 M EDTA-1% SDS and incubated at 60 C for 2 hr, then transferred to ESP solution (0.5 M EDTA (pH 9 - 9.5), 1% Na-N-lauroyl sarcosine, 1 mg/ml Proteinase K) and incubated at 50 C. After 24 hr. the blocks were transferred to fresh ESP and incubated an additional 24 hr. before storage at 4 C.

PFG analysis. All PFG separations used a 20 cm square 1% agarose gel at 15 C. Other conditions are given in the text and

figure captions. The apparatus was either the LKB pulsaphore, a 33 cm square, inhomogeneous submarine device, or a very similar 36 cm device (6). Comparable results have been obtained with a 20 cm apparatus. For identification of particular bands, the gels were blotted onto nitrocellulose after UV nicking, and the blot was cut into separate lanes. Each lane was hybridized with individual probes, and then the blot was reassembled and autoradiographed.

RESULTS

To study S. pombe chromosomes by PFG we needed to develop procedures for cell lysis without DNA damage, and we needed to modify PFG protocols to handle the extreme fragility expected for intact DNAs approaching 10 Mb in size. We found that cell lysis procedures and DNA preparations developed for S. cerevisiae (2,20) could be adapted to S. pombe (16) as described in Materials and Methods.

PFG separations are known to be sensitive to many experimental variables including pulse time, temperature, and electrical field strength (19,21). Typical operating conditions are 15 C, 25 to 120 second pulse times, and 10 Volts per cm nominal applied field for a total run time of 40 to 70 hrs. Pulse times of 90 seconds suffice to separate most of the S. cerevisiae chromosomal DNAs. Pulse times of 10-20 minutes are very effective for fractionation of Plasmodium falciparum DNAs larger than S. cerevisiae chromosomes (12). These conditions did not yield effective fractionation of S. pombe. Gels showed evidence of DNA disappearance, which could have been nuclease, but was so consistent that we suspected electrophoretic artifacts caused by the electrical field strengths and inhomogeneities used in typical PFG configurations. Such artifacts have been seen frequently before (8), especially when molecules approach or exceed the sizes of the largest S. cerevisiae chromosomes.

To test if the electrical field strength was interfering with separations of S. pombe samples, we reduced the applied electrical field to the range of 3.0 TO 4.7 V per cm. Past experience has shown that there is roughly an inverse relationship between field strength and effective pulse time (20). The effective pulse time, E , is defined as $T \times V / 10 \times D$ where T is the actual pulse time,

Figure 1. Intact chromosomal DNAs separated by PFG and then stained with ethidium. From left to right the lanes correspond to *S. pombe* 972, *P. falciparum*, *S. cerevisiae*. Conditions used were 3.0 V/cm nominal applied field with 75 min. pulse times for 162 hrs.

V is the applied voltage, and D is the length of the buffer chamber in the electrophoresis apparatus. Thus at 3.0 and 4.7 V per cm, respectively, pulse time of 75 min. and 60 min. correspond respectively to effective pulse times of 22.5 and 25 min. at 10 V per cm. To compensate for the decreased field strength, we lengthened the total run time to 150 to 162 hours. These conditions have produced excellent and reproducible fractionation of *S. pombe* DNA as shown by the examples in Figures 1 and 2.

Figure 2. Identification of *S. pombe* strain HM248 chromosomal DNAs separated by PFG. **Top.** Ethidium stained chromosomal DNAs. All lanes were loaded with identical samples, and electrophoresed at 4.7 V/cm with 60 min. pulse times for 150 hrs. **Bottom.** Analysis of the gel shown at top by hybridization with the cloned DNA probes listed in Table 1.

Wild type *S. pombe* strains show three clearly resolved bands in addition to some faster moving species that is presumably mitochondrial DNA. Strains carrying minichromosome Ch16 show an additional clearly resolved band which migrates slightly slower than the presumed mitochondrial DNA. The relative staining intensity of the two smaller chromosomal DNA bands is roughly in accord with their expected size ratios, while that of the slowest moving chromosomal DNA is often lighter than expected suggesting that not all of this species may be entering the gel under our conditions.

The mobilities of the three *S. pombe* chromosomal DNA bands are consistent with the expectations based on our estimates of their sizes. For example, the smallest *S. pombe* chromosomal DNA migrates much slower than the largest *S. cerevisiae* DNA (1.3 Mb)

Table 1. S. Pombe genomic DNA probes used for hybridization.

Plasmid	Inserted Sequence	Chromosome Location	Hybridized Band				References
			I	II	III	Ch16	
pLYS1	LYS1	I	+	-	-	-	26
pYA245	CRM1	I	+	-	-	-	27
pDB(NDA1)	NDA1	II	-	+	-	-	28,29
pTSL3	TPS13	II	-	+	-	-	23
YIp(NDA3)1	NDA3	II	-	+	-	-	18
pEE105	cenIII region	III	-	-	+	+	30
YIp32-16	ADE6	III	-	-	+	+	16
YIp10.4	rDNA	III	-	-	+	-	22
pSS160	dgIIa	I,II,III	+	+	+	+	23

and somewhat slower than the largest P. falciparum DNA (Figure 1).

To confirm that we had indeed fractionated the S. pombe chromosomes as naked DNA molecules, gels like those in Figure 2 were blotted and probed by hybridization with S. pombe cloned DNA probes assigned to known chromosomes by genetic studies. One set of results is shown in Figure 2 and summarized in Table 1. It is clear that there is a general pattern of agreement between genetic and hybridization data, indicating that PFG has indeed fractionated the S. pombe chromosomes. For example, the crm1 gene

Figure 3. DNA size dependence of the effective pulse time (see text) needed for optimal PFG separations.

is located at one end of chromosomes I and the nda1 and nda3 genes are located at the ends of chromosome V. The lys1 and tps13 genes are located at the centromere regions of chromosomes I and II respectively. Thus, taken together the hybridization results with these probes shown in Figure 3 suggest that the intact lengths of the chromosomes are preserved. Minichromosomes are derived from the pericentric region of chromosome III (16). A probe on the gene ADE6 is linked to centromere III, while the probe pEE105 contains a unique sequence near centromere III. Thus these two probes hybridize with both chromosome III and minichromosome Ch16. About 100 copies of rDNA are clustered at the distal part of chromosome III and are not present in the minichromosome (22). The hybridization patterns with these probes assign the location of chromosome III and the minichromosome. The dg sequence is a highly homogeneous repetitive sequence that exists in the centromere region of all S. pombe chromosomes (23). This probe hybridizes with all four chromosome bands.

DISCUSSION

Our success in separating S. pombe chromosomal DNAs has now extended the range of PFG to at least 9 Mb, and also provides the first rough size markers up to this range. To assist experiments to extend the range of PFG even further, we examined the dependence of the range of PFG on pulse time quantitatively. Optimal PFG separations tend to occur at the large end of the molecular weight range, just before a limiting mobility is reached. Beyond this size the molecular weight dependence of PFG mobility is lost, and a compression regions containing all larger molecules is seen. Figure 3 shows the relationship between the pulse time, and the size of the largest resolved band, which we take as a measure of the optimal separation range. The data are only rough estimates since they average results with different apparatus and different field strengths. In spite of this, it is apparent that, across more than four orders of magnitude in pulse time, and three orders of magnitude in DNA size, there is a similar scaling of optimal separation pulse time on molecular weight. The data indicate that using double inhomogeneous

electrical field conditions (6), PFG scales as the 4/3 power of the molecular weight.

The complexities of the electrical field shapes used preclude a direct interpretation of this scaling. The observed power dependence is different from predicted values but these were based on simpler experimental configurations (24-25). However, the consistency of the scaling results, across such a wide size range, encourages the extrapolation of PFG experiments to both larger and smaller DNA sizes. Indeed the smallest natural human chromosomes are only five times larger and the smallest known human chromosomes are only a factor of two larger than the largest S. pombe chromosomes. This makes us optimistic that some success should be forthcoming in attempts to separate human chromosomal DNAs electrophoretically.

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