Construction of a *Not* I restriction map of the fission yeast *Schizosaccharomyces pombe* genome

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Received November 4, 1988; Revised and Accepted February 24, 1989

ABSTRACT

Pulsed field gel electrophoresis and large DNA technology were used to construct a *Not* I restriction map of the entire genome of the fission yeast *Schizosaccharomyces pombe*. There are 14 detectable *Not* I sites in *S. pombe* 972h : 9 sites on chromosome I and 5 sites on chromosome II, while no *Not* I sites were found on chromosome III. The 17 fragments (including intact chromosome III) generated by *Not* I digestion were resolved by PFG electrophoresis. These fragments ranged in size from 4.5 kb to approximately 3.5 Mb. Various strategies were applied in determining, efficiently, the order of the fragments on the chromosomes. The genomic size measured by adding all the fragments together is about 14 Mb and the sizes of the three chromosomes are I, 5.7 Mb, II, 4.6 to 4.7 Mb, and III, 3.5 Mb. These are generally somewhat smaller than estimated previously.

INTRODUCTION

The fission yeast, *Schizosaccharomyces pombe*, is an ideal system to develop for studies of eukaryotic molecular biology (1-5). In a number of its characteristics *S. pombe* appears to be more similar to the higher eukaryotes than is the budding yeast *Saccharomyces cerevisiae* (4). An intron in a gene derived from a mammalian system (the gene encoding the SV40 small t antigen) is correctly spliced in *S. pombe* (5) and the human gene CDC2HS has been screened by genetic complementation in *S. pombe* when transformed by a human cDNA library (2). *S. pombe* chromosomes have large centromeres (6) and condense during mitosis (7). Cytoplasmic microtubules disappear prior to mitosis, followed by the formation of mitotic spindle (3). Furthermore, it was even reported that *S. pombe* chromosome III can replicate autonomously in mouse cells (1).

A complete linked bacteriophage library of the bacterium *Escherichia coli* has been finished (10). Similar fine mapping

projects are underway with the yeast Saccharomyces cerevisiae (4) and the nematode Caenorhabditis elegans (12). These maps will provide immediate access to any segment of the genome that can be defined genetically. A complete macro-restriction map of the genome of E. coli (13) and some long-range restriction maps of specific regions of eukaryotic genomes (14) have been constructed using pulsed field gel electrophoresis (PFG) (15,16) and other large DNA technologies (17). These macro-maps are very useful tools to assist the construction of finer restriction maps and overlapping clone libraries. In addition, a macro-restriction map allows the detection and analysis of chromosomal DNA rearrangements and studies of chromosomal structure and function to be carried out at the entire genome level.

Recently the three chromosomes of S. pombe were resolved by PFG (9). Here, we report the construction of a macro-restriction map of this organism as a starting point for studying the large-scale organization of the S. pombe genome and the relationship between its chromosomal structure and function. A variety of strategies and methods were used to construct a Not I restriction map of the S. pombe genome. They include use of cloned, genetically mapped genes and purified large DNA fragments as probes in hybridization experiments with complete or partially digested genomic DNA; and use of PFG purified single chromosomes. The S. pombe project should serve as a model for the construction of physical maps of other genomes.

MATERIALS AND METHODS

Strains and Plasmids

S. pombe 972h⁻ (18), a haploid wild type strain, was used for all mapping experiments. Plasmids used for Southern blotting are listed in Table 2.

Preparation of Yeast DNA Inserts and Restriction Enzyme Digests

S. pombe cells were grown to late logarithmic phase and harvested as described (19). After centrifugation, the cell pellets were washed twice with 50 mM EDTA (pH 7.5) and finally resuspended in Zymo-buffer (20 mM citrate-phosphate buffer (pH 5.6), 50 mM EDTA (pH 5.6), and 0.9 M sorbitol) containing 0.3 mg/ml Zymo-

lyase-100T (Seikagaku Kogyo); and incubated at 37 C for 2 hr. The formation of spheroplasts was checked by looking at the change of cell shape under a phase-contact microscope. A 2 ml sample of spheroplasts was mixed with 2 ml 1% low gelling temperature agarose at 50 C, distributed into 100 μ l molds and allowed to solidify on ice for about 15 min. The resulting inserts were incubated in ESP (0.5 M EDTA (pH 9-9.5), 1% Lauroyl Sarcosine and 1 mg/ml Proteinase K) for 2 days at 50 C with gentle shaking. Before restriction endonuclease digestion, DNA inserts, stored in ESP solution, were washed twice (16), 2 hr per wash, with TE buffer (10 mM Tris-HCl (pH 7.5) and 1 mM EDTA) containing 1 mM Phenylmethyl-sulfonyl Fluoride (PMSF), and then washed three times with TE buffer only. In situ restriction enzyme digestions were carried out in an Eppendorf tube at a final volume of 250 μ l for 6-12 hr. Complete digestions were obtained using an enzyme to DNA ratio (Units: μ g) of 15:1. Partial digestions were carried out at various enzyme to DNA ratios (Units: μ g) : 0.1:1, 0.3:1, 1:1, 3:1 and 10:1 for 2-3 hr.

PFG Electrophoresis

PFG electrophoresis employed the Pulsaphor apparatus (Pharmacia-LKB) with electrodes set as described elsewhere (16,17), except for Figures 2 and 3, where a home-made CHEF apparatus was used (20). A 1% agarose (Seakem LE Agarose, FMC) 20 cm square gel was run at 15 C in modified TBE buffer (100 mM Tris-HCl (pH 8.0-8.4), 100 mM boric acid and 0.2 mM EDTA). Fragments smaller than 1 Mb were resolved by PFG experiments run at 10 V/cm for 40 hr with 100 sec pulse times. Longer pulse times at lower voltages were applied to separate the fragments larger than 1 Mb (see below). Gels were then stained in 1X TBE buffer containing 1 μ g/ml ethidium bromide and destained for 30-60 min before photographing. Concatenated bacteriophage lambda DNA length standards were prepared as described elsewhere (20,23).

Southern Blotting

The DNA in the gel was nicked by exposure to 310 nm ultraviolet light, then denatured with 0.5 M NaOH and 0.5 M NaCl for 1 hr, neutralized with 0.5 M Tris-HCl, pH 7.5 and 1.5 M NaCl for 1 hr and transferred to nitrocellulose filters using 15X SSC solution

(16). Prehybridization and hybridization were carried out at the same condition (3X SSC, 10X Denhart's, 1% SDS, 100 µg/ml denatured salmon sperm DNA, 68 C). Filters were washed subsequently with 3X SSC and 1% SDS; 1X SSC and 1% SDS; 0.3X SSC and 1% SDS. Each wash was carried out at 68 C for 20 min. All the S. pombe genomic DNA clones used as probes for hybridization experiments are summarized in Table 2. Probes were radiolabeled by random oligonucleotide priming (21).

Two Dimensional PFG Experiments

An ordinary PFG gel was run for the first dimension. The gels were stained with ethidium bromide and destained as described above. DNA bands were visualized under 365 nm light and excised. Individual gel blocks (about 120 µl) containing single DNA bands were put into 1 ml solution containing 0.15 M NaCl and 30% isopropanol for 30-60 min with occasional inversion and then washed with 5 ml TE buffer twice at room temperature for 2 hr each. Restriction enzyme digestion and PFG electrophoresis with these gel blocks were then carried out as done with the original DNA inserts described above.

Labeling of Not I Fragments by End-filling

Two µg of S. pombe DNA was digested with 30 Units of Not I in a 100 µl volume reaction containing 100 mM NaCl, 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM dithiothreitol, 4 Units Klenow polymerase (2 U/µg DNA) and 50 µCi ³²P-dGTP (25 µCi/µg DNA) at 37 C for 6 hr and then treated with ESP solution as described above. Two tenths µg labeled DNA samples were loaded onto both PFG gels and ordinary gels. To detect the small fragments which may diffuse out of the agarose inserts during the digestion and labeling, liquid portion of the reactions was phenol extracted. The DNA was ethanol precipitated and loaded along with the samples in the inserts.

Pre-association of Labeled Not I Fragments with Cold S. pombe DNA

The labeled S. pombe Not I fragment DNA was mixed with cold S. pombe DNA at various ratios (labeled DNA:cold DNA (ng:µg): 1:0.4, 1:2 and 1:10); boiled for 10 min and then kept at 68 C for 2 hr before being added to the hybridization bag.

Figure 1. PFG separation of the fragments of *Schizosaccharomyces pombe* DNA produced by digestion with various restriction enzymes. PFG electrophoresis was carried out at 10 V/cm for 40 hr using 100 sec pulse times, and the gel was stained with ethidium bromide. Lanes 1 and 10 contain intact *S. cerevisiae* chromosomal DNAs. Lanes 2 and 9 are multimers of 48.5 kb lambda DNA used as length standards. Lanes 3 to 8 contain wild type *S. pombe* 972h chromosomal DNA digested with *Mlu* I, *Not* I, *Not* I plus *Sfi* I, *Sfi* I, *Sal* I and *Xho* I, respectively.

RESULTS

Generation of Megabase Fragments from *S. pombe* Chromosomal DNA

Digests of *S. pombe* genomic DNA with various restriction enzymes: *Mlu* I, *Not* I, *Sac* II, *Sal* I, *Sfi* I, *Sma* I and *Xho* I, were analyzed by PFG. Of the enzymes tested only the two known 8-base specific nucleases (22) *Sfi* I and *Not* I yielded a small number of relatively large fragments. A typical example of an ethidium bromide stained PFG separation resolving fragments up to 1 Mb is shown in Figure 1. The *Not* I digest was selected for further analysis because its pattern was the simplest and the best resolved. Detailed analysis shows that *S. pombe* has 14 detectable *Not* I sites: 9 sites on chromosome I and 5 sites on chromosome II. Thus these two chromosomes are cut into 10 and 6 fragments, respectively. Chromosome III has no *Not* I sites and remains intact following digestion with the restriction enzyme *Not* I (see below). The *Not* I fragments range in size from approximately 3.5

Figure 2. *S. pombe* strain 927h⁻ chromosomal DNA was digested with *Not* I and electrophoresed in an agarose gel by a home-made CHEF apparatus. Pulsed field electrophoresis was carried out at 7.0 V/cm for 24 hr using 45 sec (lanes 1 and 2) and 90 sec (lane 3) pulse times, or at 4.0 V/cm for 96 hr using a 600 sec pulse time (lane 4). The gel was stained with ethidium bromide. A - P designate bands for *Not* I fragments. In this figure, the size of the lambda DNA monomer (lane 1) is 42.5 kb.

Mb to 4.5 kb, and are designated, respectively, fragment A through Q. Multiple pulse times are required to resolve the entire set of fragments, whose sizes cover a 800-fold range (see Figures 2 and 3). For example, two unresolved doublets (G and H; I and J) in a gel with a 90 sec pulse time can be separated by using a 60 sec pulse time. The largest three fragments A, B, and C can only be resolved by exceedingly long pulse times (600-4500 sec).

There is 800-fold size difference among the *S. pombe Not* I fragments. Thus, small genomic fragments may be missed if one simply examines ethidium bromide stained gels because ethidium bromide staining intensity is proportional to the size of DNA fragments. In addition, small DNA fragments diffuse out of the agarose inserts. The smallest fragment (Q), can not be detected by ethidium bromide staining since only 0.2-0.4 μ g DNA/lane is loaded in PFG experiments, can be detected when the *Not* I fragments were end-labeled with ³²P-dGTP using Klenow polymerase as described in Materials and Methods (data not shown).

Figure 3. Identification of individual *S. pombe* Not I fragments by hybridization with cloned DNA probes. Odd lanes are ethidium stained bands; even lanes are autoradiograms. PFG was carried out as described in the legend to Figure 2. Pulse times were 600 sec for lanes 1-4; 90 sec for lanes 5-6; and 45 sec for lanes 7-8. Hybridization probes were *dg*, lane 2; *top2*, lane 4; *crml*, lane 6; and *top1*, lane 8.

Not I Fragment Sizes

Sizes of the fragments are shown in Table 1. The size of fragments smaller than 1,200 kb was determined by comparing the mobility of the DNA fragments with that of DNA size standards composed of tandemly annealed lambda DNA oligomers electrophoresed in adjacent lanes (16,23). Routinely such size markers extend well beyond 1 Mb in size, but it is usually difficult to resolve adjacent bands unequivocally above the 24-mer of lambda DNA. There are three Not I fragments (A, B, and C) with sizes above 1,200 kb. Their size was determined by several methods.

First, partial Not I digests of *S. pombe* chromosome I were used to provide size standards in the 530-5,700 kb range and these were then used to determine the sizes of fragments A, B, and C shown in Table 1. Since the largest Not I fragment of chromosome I is 1200 kb, the size of this chromosome can be determined just by adding the sizes of all of its constituent fragments. Then partial digests, like that shown in Figure 4, once interpreted, yield bands of known size up to the full length of the chromosome (44). Second, new Not I restriction sites were

Table 1. Sizes of the Not I fragments seen in a total digest of S. pombe 972 h. The sizes (except for fragments A, B and C) were determined by comparing the mobility of DNA bands with that of DNA size standards composed of tandemly annealed lambda DNA oligomers electrophoresed in adjacent lanes. Sizes of fragments A, B and C were determined by comparison with a partial Not I digest of S. pombe chromosome I.

<u>Fragments</u>	<u>Size (kb)</u>	<u>Chromosome</u>
A	3,500	III
B	2,000	II
C	1,525	II
D	1,200	I
E	1,010	I
F	900	I
G	625	II
H	600	I
I	530	I
J	500	I
K	470	I
L	380	I
M	240	II
N	175	II
O	135	I
P	88	II
Q	4.5	I

introduced into fragments A, B, and C on the chromosomes by site-specific inte-gration to generate smaller Not I fragments, which could then be sized by comparison with lambda size standards. Adding these smaller fragments together yields consistent sizes of fragments A, B, and C (Chikashige, Y. et al., unpublished data). Third, chromosome III, i.e. fragment A, was purified by PFG electrophoresis and digested by the restriction enzyme Eag I, which recognizes the inner 6 nucleotides of the Not I site. The fragments generated by Eag I digestion are all smaller than 250 kb. Densitometer scanning was used to estimate the number of fragments in unresolved bands. Adding all these fragment sizes together produced an estimate for the size of fragment A of 3.5 Mb, consistent with that obtained by the first two methods.

Physical Mapping of Not I Fragments

Direct Southern blotting was used to determine the correspondence between individual Not I fragments and the previously known S. pombe genetic map. About 30 clones were used as DNA probes in

Table 2. Probes Used for Hybridization

Gene	Clone	Hybridized Band(s)	Reference
<u>ade5</u>	Ylp(ade5)	A	Niwa, O. (pers. comm.)
<u>ade6</u>	pAS1	A	Kohli, J. (pers. comm.)
<u>ura4</u>	pura4	A	Nurse, P. (pers. comm.)
<u>cdc2</u>	pcdc2.19	B	26
<u>mat2</u>	psm2	B	27
<u>mei3</u>	pDB(me13)2	B	28
<u>nda1</u>	pDB(NDA1)1	B	29
<u>top2</u>	pTOP2-1	B	30
<u>nda2</u>	pDB(NDA2)1	C	31
<u>nda3</u>	YIP(NDA 3)1	C	32
<u>mes1</u>	pDB(mes1)1	D	Shimoda, C. (pers.comm.)
<u>aro3</u>	pFNA2302	E	33
<u>mei2</u>	pDB(me1 2)2	E	34
<u>nuc2</u>	pUC(Nuc 2)2-2	E	35
<u>crml</u>	HIP(crml)	F	Adachi, Y. <u>et al.</u> , to be published
<u>cdc10</u>	pcdc10.3	G	36
<u>top1</u>	pUC18(Top 1)	G	37
<u>sds22</u>	pHR140	H	Ohkura, T. <u>et al.</u> , to be published
<u>sds23</u>	pHR150	H	<u>ibid.</u>
<u>ural</u>	pFYM225	J	38
<u>cyh1</u>	pcy1	K	6
<u>lys1</u>	plys1	K	39-40
<u>cdc13</u>	pcdc13	N	<u>ibid.</u>
<u>cdc25</u>	pCDC25-58	O	41
<u>atb2</u>	pYA113	P	42
<u>tel</u> ¹	pSPT16	A,C,I,L,M	43
<u>dgII</u> ²	pSS166	A,B,K	6

1--telomeric DNA.

2--centromeric DNA.

hybridization experiments (Table 2). They all are unique in the genome except the telomeric and centromeric probes. Figure 3 shows an example of typical results with the centromeric probe. Altogether, the available probes allowed the unequivocal identification of 13 of the 17 bands. They also identified, collectively, 5 bands which hybridized with the telomeric probe (A, C, I, L, M) and 3 bands which hybridized with the centromeric probe (A, B, K).

Partial digestion strategies were used for identification of fragments neighboring those already assigned (13). Such strate-

Figure 4. An example of the use of partial digestion for mapping neighboring *Not* I fragments. A blot of a partial *Not* I digest of *S. pombe* 972h chromosomal DNA was probed with the *cdc25* gene on chromosome I, which was assigned to fragment O (135 kb). From left to right the digest is progressively more complete (Enzyme Units: μ g of DNA: 0.1:1, 0.3:1, 1:1, 3:1 and 10:1; 37 C for 3 hr). The pattern of hybridization shows several intermediates, 515 kb, 635 kb, 1015 kb, 1.8 Mb and 2.2 Mb, besides the complete digest band of 135 kb. The 1.8 Mb and 2.2 Mb fragments can not be well resolved under the conditions used for this gel, thus a wider hybridization band was seen at this region. We interpret the 515 kb band as fragment O plus fragment L (380 kb); the 635 kb band as fragment O plus fragment J (500 kb); the 1015 kb band as fragment O plus fragments L and J; the 1.8 Mb band as fragment O plus fragments J and D (1200 kb); and the 2.2 Mb band as fragment O plus fragments L, J and D. The interpretation of the partial digest shown at the top of the figure was further confirmed by hybridization with an *S. pombe* telomeric probe (which hybridized to fragment L) and with the *ura1* gene (which hybridized to fragment J).

Figure 5. Not I digestion of single chromosomes purified by PFG electrophoresis. This gel was run at 10 V/cm for 40 hr using 100 sec pulse times. Lanes 1 and 7 are lambda size standards. Lanes 2 and 6 are total *S. pombe* chromosomal DNA digested with Not I. Lanes 3, 4 and 5 are purified *S. pombe* chromosome I, II and III, respectively, digested with Not I. Since there is no Not I site on chromosome III, only a very large fragment can be seen in lane 5 near the top of the gel. By comparing lane 2 with lanes 3 and 4, it is easy to tell that fragments E, F, H, I, J, K, L and O are from chromosome I while fragments G, M, N and P are from chromosome II.

gies are necessary when a probe is not available that hybridizes to a particular fragment and are an efficient method for finishing maps and proving that particular fragments are actually adjacent. In order to see all the intermediates in a partial digestion panel, pilot experiments using different digestion times and different ratios of enzyme to DNA are necessary. An example of a successful and informative partial digest analysis is shown in Figure 4. This gel assigned four Not I fragments covering more than 2,200 kb of chromosome I. The order of fragments from the telomere is L, O, J, D.

Since *S. pombe* has three chromosomes, 3 and 6 fragments are expected, respectively, when PFG gels are probed with cross hybridizing centromeric and telomeric probes. Only 5 fragments, however, are found when probed with a telomeric sequence. This is due to the absence of Not I sites on chromosome III, resulting in only one intact chromosome band (see below). By subjecting purified individual chromosomes to Not I digestion, the same

Figure 6. Evidence that chromosome III contains no *Not* I sites. A. An ethidium bromide stained gel. PFG electrophoresis was run at 3 V/cm for 120 hr using 3600 sec pulse times. Lanes 1 and 7 are intact *S. pombe* chromosomes. Lanes 2 to 6 are *Not* I digestions of *S. pombe* chromosomes with decreasing amounts of enzyme (Units: μ g: 12.5:1, 7.5:1, 3:1, 1:1, 0.1:1 at 37 C for 8 hr). Lanes 8 to 11 are *Sfi* I digestions of *S. pombe* chromosomes with decreasing amounts of enzyme (Units: μ g: 12.5:1, 1:1, 0.1:1, 0.03:1; at 50 C for 8 hr). B. Southern hybridization to the gel shown in part A using a probe composed of three genes (*ura4*, *ade5* and *ade6*) from the ends and middle respectively of *S. pombe* chromosome III.

probes can be used to assign the centromeric or telomeric bands of each chromosome unambiguously. First, the three chromosomes were separated by PFG electrophoresis, and agarose slices containing these were excised as described in Materials and Methods. Then the single chromosomes were digested *in situ* and loaded onto a second gel. The resulting pattern of fragments clearly demonstrates chromosome-specific bands as shown by the example in Figure 5. This approach was used to determine that telomeric fragments L and I are located on chromosome I, and fragment M is located on chromosome II. Partial digestion experiments were carried out to locate telomeric fragments to specific chromosome ends using nearby genes as probes. These results are consistent with Southern hybridizations using the three unfractionated chromosomes. The sizes of particular fragments from the digests of both PFG-purified single chromosomes and the original DNA inserts are the same, indicating that chromosomal DNAs remain intact dur-

Figure 7. Alignment of the genetic map with the Not I restriction map of the *S. pombe* genome. Listed next to each Not I fragment are the probes and methods used to assign its location.

ing PFG electrophoresis. This approach provides a general method to distinguish any ambiguous fragments from different chromosomes.

The results in Figure 5 show only a single ethidium stained band for *S. pombe* chromosome III. This suggests that there are no *Not* I sites on this chromosome. However, chromosome III is running in the compression region of the gel where all DNAs larger than 1 Mb comigrate. Longer pulse time PFG analyses were performed to confirm the absence of *Not* I sites on chromosome III (Figure 6). In Figure 6A, both *Not* I and *Sfi* I digestions produce a fragment which appears to migrate as intact chromosome III. However, hybridization with three chromosome III specific probes reveals that *Sfi* I cleaves chromosome III while *Not* I leaves it uncut (Figure 6B). Hence, the fragment that runs as intact chromosome III seen in the *Sfi* I digestion must come from chromosome I or chromosome II. The *Sfi* I fragments seen in Figure 6B allow a minimum estimate of the size of chromosome III of 3.5 Mb, which turns out to be consistent with other results described previously. One weak hybridization band can be seen at the bottom of Figure 6B lane 2 and lane 3. We believe this band is due to random degradation of chromosome III by contaminated endonucleases during the *Not* I digestion.

Unmapped large DNA fragments may be used directly as hybridization probes in organisms with small genomes. Two *S. pombe* *Not* I fragments were mapped using this strategy. A total *Not* I digest of *S. pombe* was fractionated by PFG. Individual fragments were excised from the gel; DNA was eluted from the agarose and labeled with ³²P-dCTP by random oligonucleotide priming. This DNA probe was then used in hybridization to analyze samples partially digested with the same enzyme, *Not* I. The labeled fragment was subjected to a pre-association step (see Methods), using cold *S. pombe* DNA, to block hybridization of any repetitive sequences on the probe to genomic DNA immobilized on nitrocellulose. The sizes of the partial digestion fragments detected allow one to deduce the sizes of neighboring fragments. Usually, this is sufficient to locate the labeled fragment unequivocally. The locations of fragments H and Q were defined in this way, and both map to chromosome I. Recently, two genes, *sds22* and *sds23*,

which genetically map to the fragment H region, were cloned (Ohkura, H. et al, unpublished results). Use of these clones as hybridization probes confirmed the interpretation of the partial digest pattern seen with labeled fragment H.

DISCUSSION

A Not I restriction map of the S. pombe genome was completed using a variety of large DNA strategies (Table 1 and Figure 7). Most of the Southern blotting data were double checked at both Columbia University and Kyoto University. The fragment sizes added together indicate that the size of the S. pombe genome is about 14 Mb. This is somewhat smaller than the average of the size estimates obtained by previous genetic and cytological studies (24-26). The sizes determined for the three S. pombe chromosomes are I, 5.7 Mb, II, 4.6 to 4.7 Mb and III, 3.5 Mb. The two large chromosomes are thus quite a bit smaller than earlier size estimates (6,9,24).

This restriction map is the first complete restriction map for any eukaryotic organism. Both high and low resolution restriction maps have been completed for E. coli (10,13) which has a 4.7 Mb genome. The experiments described in the text show that a low resolution restriction map may easily be completed for an organism having a genome several times the size of E. coli. Thus, the approaches discussed here can be applied to a wide variety of organisms.

The analysis of large DNA molecules requires that their sizes be known. Lambda phage concatemers can only be used to size molecules up to about 1.2 Mb (23). The S. pombe chromosomes themselves can now serve as size standards for DNA molecules up to the size of chromosome I (5.7 Mb).

The existing Not I map will allow immediate access to any segment of the genome that can be defined genetically or biochemically. It can be used to indicate the occurrence of large DNA rearrangements. We are currently constructing an Sfi I map of the S. pombe genome. When combined with Not I results, the physical map of this organism will be more detailed and more useful. Any particular region of interest in S. pombe can now be easily characterized by finer restriction mapping using indirect end

labeling approaches from ends of Not I fragments. Probes for such experiments can be generated from linking clones (17) by using the half linking clone specific to the fragment.

The simplicity of the Not I map of S. pombe suggests that this organism would be an excellent host for the cloning and characterizing large mammalian DNA fragments, because it might be relatively easy to distinguish any additional DNA pieces from the normal S. pombe genome. In fact, the recent demonstration that S. pombe chromosome III can replicate in mammalian cells (1) suggests this organism may be useful for developing a mammalian large DNA cloning shuttle system.

ACKNOWLEDGEMENT

We are grateful to Drs. D. Beach, P. Fantes, J. Kohli, P. Munz, P. Nurse, H. Schmidt, C. Shimoda, J. Szostak, K. Wolf and M. Yamamoto for supplying many of the generous DNA probes for the hybridizations and the S. pombe strains. We would like to thank Stephanie Klco and Steve Cooper for skilled technical assistance. This work was supported by grants from the NIH, GM 14825; the NCI, CA 39782; the DOE, DE-F602-87ER-GD852; the MacArthur Foundation; Pharmacia-LKB; and the Ministry of Education, Science and Culture and the Science and Technology Agency of Japan.

REFERENCES

1. Allshire, R.C., Cranston, G., Gosden, J.R., Maule, J.C., Hastie, N.D. and Fantes, P.A. (1987) *Cell* 50, 391-403.
2. Lee, M.G. and Nurse, P. (1987) *Nature* 327, 31-35.
3. Kohli, J. (1987) *Current Genetics* 11, 575-589.
4. Russell, P. and Nurse, P. (1986) *Cell* 45, 781-782.
5. Kaufer, N.F., Simanis, V. and Nurse, P. (1985) *Nature* 318, 78-80.
6. Nakaseko, Y., Adachi, Y., Funahashi, S.-I., Niwa, O. and Yanagida, M. (1986) *EMBO J.* 5, 1011-1021.
7. Uemura, T., Ohkura, H., Adachi, Y., Morino, K., Shiozaki, K. and Yanagida, M. (1987) *Cell* 50, 917-925.
8. Hagan, I.M. and Hyams, J.S. (1988) *J. Cell Sci.* 89, 343-357.
9. Smith, C.L., Matsumoto, T., Niwa, O., Klco, S., Fan, J.-B., Yanagida, M. and Cantor, C.R. (1987) *Nucleic Acids Res.* 15, 4481-4489.
10. Kohara, Y., Akiyama, K. and Isono, K. (1987) *Cell* 50, 495-508.
11. Olson, M., Dutchik, J.E., Graham, M.Y., Brodeur, G.M., Helms, C., Frank, M., MacCollin, M., Scheinman, R. and Frank, T. (1986) *Proc. Natl. Acad. Sci. USA.* 83, 7826-7830.
12. Coulson, A., Sulston, J., Brenner, S. and Karn, J. (1986) *Proc. Natl. Acad. Sci. USA.* 83, 7821-7825.

13. Smith, C.L., Econome, J.G., Schutt, A., Klco, S. and Cantor, C.R. (1987) *Science* **236**, 1448-1453.
14. Lawrance, S.K., Smith, C.L., Srivastava, R., Cantor, C.R. and Weissman, S. (1987) *Science* **235**, 1387-1390.
15. Schwartz, D.C., Saffran, W., Welsh, J., Haas, R., Goldenberg, M. and Cantor, C.R. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 189-195.
16. Smith, C.L. and Cantor, C.R. (1987) In Wu, R. (ed), *Methods in Enzymology*, Academic Press, New York, Vol. **155**, pp.449-467.
17. Smith, C.L., Lawrance, S.K., Gillespie, G.A., Cantor, C.R., Weissman, S.M. and Collins, F.S. (1987) In Gottesman, M. (ed), *Methods in Enzymology*, Academic Press, New York, Vol. **151**, pp.461-489.
18. Gutz, H., Heslot, H., Leupold, U. and Loprieno, N. (1974) In King, R.C. (ed), *Handbook of Genetics*, Plenum Press, New York, Vol. **1**, pp.395-446.
19. Niwa, O., Matsumoto, T. and Yanagida, M. (1986) *Mol. Gen. Genet.*, **203**, 397-405.
20. Chu, G., Vollrath, D. and Davis, R.W. (1986) *Science* **234**, 1582-1585.
21. Feinberg, A.P. and Vogelstern, B. (1983) *Analytical Biochemistry* **132**, 6-13; *ibid* **137**, 266-267.
22. Qiang, B.-Q. and Schildkraut, I. (1987) In Wu, R. (ed), *Methods in Enzymology*, Academic Press, New York, Vol. **155**, pp.15-21.
23. Mathew, M.K., Smith, C.L. and Cantor, C.R. (1988) *Biochemistry*, in press.
24. Bostock, C.J. (1970) *Exp. Cell Res.* **60**, 12-26.
25. Nurse, P. and Thuriaux, P. (1977) *Exp. Cell. Res.* **107**, 365-375.
26. Beach, D., Durkacz, B. and Nurse, P. (1982) *Nature* **300**, 706-709.
27. Beach, D.H. (1983) *Nature* **305**, 682-688.
28. Shimoda, C. and Uehara, M. (1985) *Mol. Gen. Genetics* **201**, 353-356.
29. Yanagida, M., Hiraoka, Y., Uemura, T., Miyake, S. and Hirano, T. (1986) In Hicks, J. (ed), *Yeast Cell Biology*. UCLA Symposia on Molecular and Cellular Biology, Alan R. Liss, New York, Vol. **33**, pp. 279-297.
30. Uemura, T. and Yanagida, M. (1986) *EMBO, J.* **5**, 1003-1010.
31. Toda, T., Adachi, Y., Hiraoka, Y. and Yanagida, M. (1984) *Cell* **37**, 233-242.
32. Hiraoka, Y., Toda, T. and Yanagida, M. (1984) *Cell* **39**, 349-358.
33. Nakanishi, N. and Yamamoto, M. (1984) *Mol. Gen. Genet.* **195**, 164-169.
34. Shimoda, C., Uehira, M., Kishida, M., Fujioka, H., Iino, Y., Watanabe, Y. and Yamamoto, M. (1987) *J. Bact.* **169**, 93-96.
35. Hirano, Y., Hiraoka, Y. and Yanagida, M. (1988). *J. Cell. Biol.* **106**, 1171-1180.
36. Aves, S.J., Durkacz, B.W., Carr, A. and Nurse, P. (1985) *EMBO J.* **4**, 457-463.
37. Uemura, T., Morino, K., Uzawa, S., Shiozaki, K. and Yanagida, M. (1987) *Nuc. Acids Res.* **15**, 9727-9739.
38. Sakaguchi, J. and Yamamoto, M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7819-7823.
39. Beach, D., Piper, M. and Nurse, P. (1982) *Mol. Gen. Genet.*, **187**, 326-329.

40. Beach, D.H., Rodgers, J. and Gould, J. (1985) Curr. Genet. 10, 297-311.
41. Russell, P. and Nurse, P. (1986) Cell 45, 145-153.
42. Adachi, Y., Toda, T., Niwa, O. and Yanagida, M. (1986) Mol. Cell. Biol. 6, 2168-2178.
43. Sugawara, N. and Szostak, J.W. (1986) Yeast 2 (supp), 373.
44. Ringquist, S., Fan, J-B. and Smith, C.L. in preparation.