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# Alignment of *Sfi* I sites with the *Not* I restriction map of *Schizosaccharomyces pombe* genome

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## ABSTRACT

**A *Sfi* I restriction map of the fission yeast *Schizosaccharomyces pombe* genome was aligned with the *Not* I restriction map. There are 16 *Sfi* I sites in the *S. pombe* genome. Three *Sfi* I sites are on chromosome III which is devoid of *Not* I sites. The sizes of the entire genome and individual chromosomes, calculated from the *Sfi* I fragment sizes, are consistent with that calculated from the *Not* I fragment sizes. The *Sfi* I map provides greater physical characterization of the *S. pombe* genome and further validates the use of *S. pombe* chromosomal DNA as size standard. These maps have allowed detection of polymorphism on all three chromosomes.**

## INTRODUCTION

The fission yeast, *Schizosaccharomyces pombe*, is a well characterized single-celled eukaryote. More than 460 genes have been defined by classical mutation analysis and by molecular cloning (1, 2). Over 270 genes have been genetically mapped (1, 2). In recent years, *S. pombe* has been developed into a convenient system for molecular biology studies (3–8). For instance, intensive cytological and molecular experiments have focused on the cell division process in this organism (9–13).

*S. pombe* has three chromosomes, which can be resolved by pulsed field gel (PFG) electrophoresis (8, 14). The sizes of the chromosomes I, II, and III are 5.7 megabase pairs (Mb), 4.7 Mb and 3.5 Mb, respectively (5). A *Not* I restriction map had been constructed for the *S. pombe* genome (5). However, no *Not* I sites are found on chromosome III. To further characterize the *S. pombe* genome physically, we constructed a *Sfi* I restriction map and aligned it with the previously constructed *Not* I map (5).

## MATERIALS AND METHODS

### Strain and cloned DNA sequences

*S. pombe* 972h$^-$ (15), a haploid wild type strain, was used for all the mapping experiments in this study. Most of the DNA clones used as probes in Southern blotting experiments were described previously (5). The *Not* I linking clone, pNOT105 and NUC2 DNA probe were generous gifts from Dr. M. Yanagida (Kyoto University).

### Preparation of rDNA probe

Genomic organization and DNA sequences of the major (25S-5.8S-18S) rDNA genes of *S. pombe* have been reported (16). The polymerase chain reaction (PCR) was used to make rDNA probe from the *S. pombe* genomic DNA. Amplification reactions were performed in volumes of 100 μl containing 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 250 μM each of dATP, dGTP, dCTP and dTTP, 0.1 μM primers, 30 ng of genomic DNA and 2.5 Units of *Taq* DNA polymerase (Perkin Elmer Cetus). Amplification was performed in a Perkin Elmer Cetus DNA Thermal Cycler programmed for 30 cycles of 30 second at 96°C, 1 second at 40°C, 2 min at 50°C, 1 min at 60°C and 1 min at 72°C. The primers' sequences (15-mers) were chosen from 18S rDNA for the forward primer: 5'ATGCCCTTAGATGTT3' and 5.8S rDNA for the reverse primer: 5'GTAGAACCCAAAGGC3'.

### Cloning of unique DNA sequence from chromosome I

No unique DNA sequences have been isolated previously from the right arm of chromosome I (1, 2). Therefore, DNA sequences from this region were cloned using a direct physical approach. At first, *S. pombe* chromosomal DNA was digested with *Not* I and fractionated by PFG. Then, the *Not* I-fragment **I**, which was mapped to the right arm of chromosome I was excised and digested with the restriction enzyme *EcoR* I as described elsewhere (5). The resulting *EcoR* I-fragments were subcloned into the *EcoR* I site of plasmid pGEM-blue (Promega). Plasmid pF7 was isolated as one of these anonymous clones. Hybridization experiments confirmed that pF7 contains a unique DNA sequence from *S. pombe Not* I-fragment **I**. In the same way, two unique anonymous DNA sequences were isolated from *Not* I-fragment **H** as recombinant plasmids pNotH-5 and pNotH-7.

### Manipulation and analysis of genomic DNA

Concatenated bacteriophage lambda DNA length standards were prepared as described elsewhere (17). A monomer of the lambda DNA is 48.5 kilobase pairs (kb). Protocols for the preparation of yeast DNA agarose inserts and enzyme digestion with single restriction enzyme were described previously (18). For *Not* I-*Sfi* I double digestion, chromosomal DNA was digested first with *Sfi* I in a medium-salt reaction buffer (50 mM NaCl, 10 mM

Tris-HCl (pH 7.5), 10 mM $MgCl_2$ and 1 mM dithiothreitol) for 5–10 hours at 50°C. Then, the reaction buffer was adjusted to 100 mM NaCl and 50 mM Tris-HCl, *Not* I was added, and the sample was incubated for 5–8 hours at 37°C. To drive the reaction to completion, the ratio of enzyme Units : μg of DNA samples was set at 15–20 : 1. The restriction enzymes were removed from the DNA inserts by proteinase K treatment before samples were loaded onto a gel (18).

The PFG conditions needed to separate *S. pombe Sfi* I fragments have been previously established in this laboratory (5, 18, 19). Usually, a 100 second pulse time at 10 V/cm for 40 hours was used to separate DNA molecules ranging in size from 50 kb to 1200 kb. Separation of larger DNA molecules required a longer pulse time, a longer running time and a lower field strength (18, 19). The gels were run at 14–15°C in modified TBE buffer (100 mM Tris-HCl, pH 8.0–8.4, 100 mM boric acid and 0.2 mM EDTA). All the DNA samples were electrophoresed in 1% agarose (SeaKem LE Agarose, FMC). The gels were stained in distilled water containing 1 μg/ml ethidium bromide for 10 minutes and destained in the TBE running buffer for about 1 hour before photographing.

### DNA hybridization

Southern blotting experiments were carried out as described previously (5). PFG fractionated DNA in agarose gels was nicked by exposuring to a UV light before being transfered onto nylon membranes (19). Prehybridization and hybridization were carried out at 68°C under the same condition (3×SSC, 10×Denhardt's solution, 1% Sodium Dodecyl Sulfate (SDS) and 100 μg/ml denatured salmon sperm DNA). DNA probes were radiolabeled by the random oligonucleotide priming method (20).

### Densitometric scanning

Autoradiographs were scanned on a Pharmacia-LKB Ultrascan laser densitometer (model 2202). Peak intensity was calculated using a Pharmacia-LKB integrator (model 2221). Hybridized membranes were directly scanned on a PhosphorImager (Molecular Dynamics) using software ImageQuant, version 2.0.

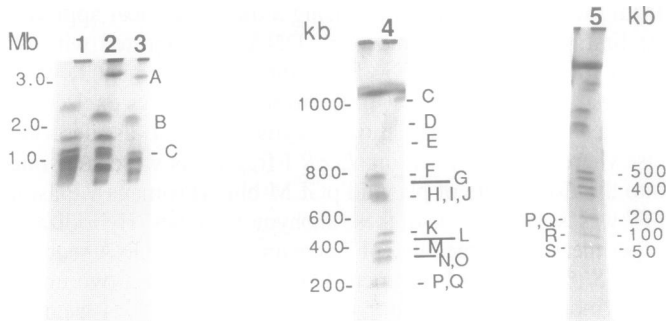



**Figure 1.** *S. pombe Sfi* I fragments detected by staining with ethidium bromide. *S. pombe* strain 972h⁻ chromosomal DNA was digested with the restriction enzyme *Sfi* I and electrophoresed in an agarose gel on a LKB Pulsaphor apparatus. PFG electrophoresis was carried out at a field strength of 3 V/cm for 140 hr using 1800 second pulse time (lane 1–3), at a field strength of 10 V/cm for 48 hr using 100 second pulse time (lane 4) and at a field strength of 10 V/cm for 40 hr using 60 second pulse time (lane 5). The gel was stained with ethidium bromide. *Sfi* I fragments are designated **A-S**. Lanes 3, 4 and 5 contain *S. pombe* DNA digested with *Sfi* I. Lane 1 contains intact *Saccharomyces cerevisiae* chromosomal DNA. Lane 2 contains *S. pombe* DNA digested with *Not* I.

## RESULTS

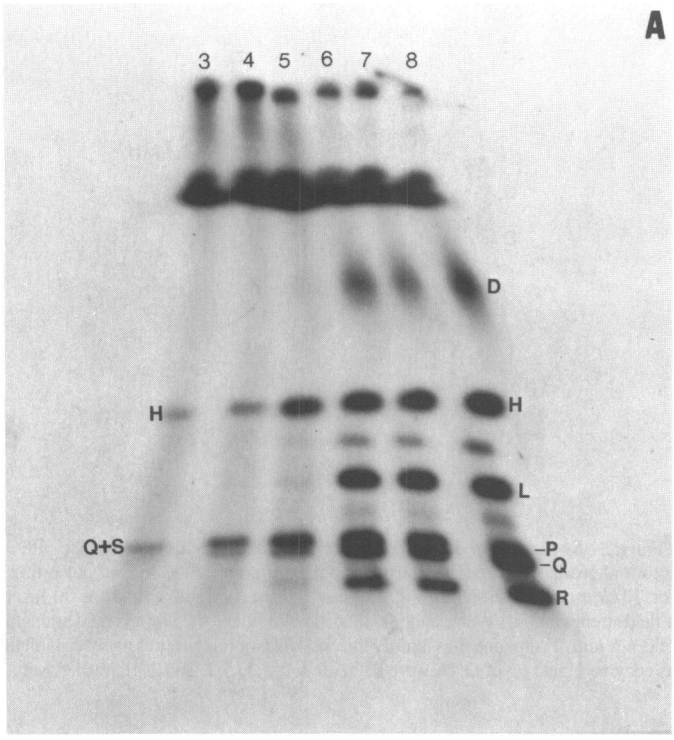
### Digestion of *S. pombe* chromosomal DNA with restriction enzyme *Sfi* I

Intact *S. pombe* chromosomal DNA, purified in agarose blocks, was digested with the restriction enzyme *Sfi* I and fractionated by PFG (Figure 1). A total of 19 *Sfi* I fragments were detected for the *S. pombe* genome with ethidium bromide staining: 9 fragments from chromosome I, 6 fragments from chromosome II and 4 fragments from chromosome III (see below). These *Sfi* I fragments range in size from 65 kb to 2.9 Mb. The fragments are designated **A** through **S**, beginning with the largest. The sizes and chromosomal locations of the *Sfi* I fragments are summarized in Table 1. Note that *Sfi* I-fragment **D**, containing the major rDNA repeat (see below), always migrated diffusively (lane 4, Figure 1, and lanes 6–8, Figure 2A). This probably reflects heterogeneity in the number of rDNA repeats. The experiments described here would have missed very small *Sfi* I fragments since (1) ethidium bromide staining intensity is proportional to DNA size and (2) small DNA fragments may have diffused out of the agarose inserts during manipulation.

The restriction enzyme *Sfi* I recognizes the DNA sequence 5′GGCC$(N)_5$GGCC3′. The occurrence of different DNA

**Table 1.** Chromosomal assignments of *Sfi* I and *Not* I fragments.

| Fragments | Sizes (kb) | Chromosomes |
|---|---|---|
| *Sfi* I: | | |
| A | 2900 | II |
| B | 1900 | III |
| C | 1035 | I |
| D | 915 | III |
| E | 850 | I |
| F | 770 | I |
| G | 750 | I |
| H | 705 | I |
| I | 705 | I |
| J | 705 | II |
| K | 480 | I |
| L | 446 | II |
| M | 383 | III |
| N | 350 | II |
| O | 325 | I |
| P | 242 | III |
| Q | 218 | II |
| R | 110 | I |
| S | 65 | II |
| total: 13881 kb (I: 5730 kb; II: 4684 kb; III: 3440 kb.) | | |
| *Not* I: | | |
| A | 3500 | III |
| B | 2000 | II |
| C | 1525 | II |
| D | 1200 | I |
| E | 1010 | 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 |
| total: 13878 kb (I: 5725 kb; II: 4653 kb; III: 3500 kb.) | | |



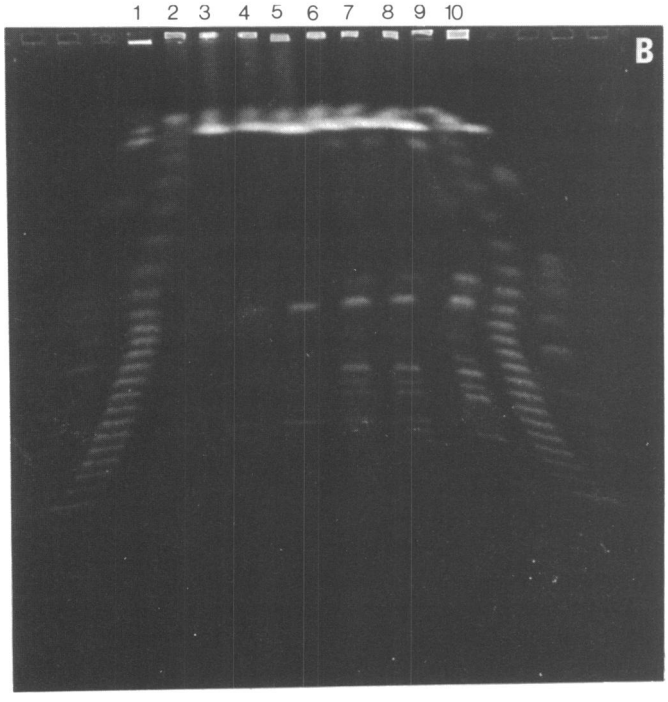




**Figure 2.** Identification of telomeric *Sfi* I fragments. PFG electrophoresis was carried out at a field strength of 10 V/cm for 40 hours using 100 second pulse time. The gel was stained with ethidium bromide as described in Materials and Methods. Lanes 1 and 10 contain intact *S. cerevisiae* chromosomal DNAs. Lanes 2 and 9 contain lambda DNA size standards. Lanes 3–8 contain *S. pombe* DNA digested with the restriction enzyme *Sfi* I. From left to right, the digest is progressively more complete. The enzyme Units: μg of DNA were 0.03:1, 0.1:1, 0.3:1, 1:1, 3:1, and 10:1, in lanes 3–8, respectively. (A) Hybridization of the ethidium bromide stained PFG gel shown in (B) with a telomeric probe (for detail, see text) indicated six telomeric *Sfi* I-fragments, **D**, **H**, **L**, **P**, **Q** and **R**, and several *Sfi* I digestion intermediates.

sequences in the middle of the recognition sites leads to very different reaction kinetics for *Sfi* I cleavage events (21). Thus, it is difficult to achieve a complete *Sfi* I digestion. For instance, the *Sfi* I cleavages at the **C-E** and **I-O** fragment junctions are much slower than the cleavages at most of the other *Sfi* I sites (see below). *Sfi* I digestion intermediates are detected as faint extra DNA bands in the PFG gels (Figure 2B).

### Sizes of the *Sfi* I fragments

The sizes of most of the *Sfi* I fragments were determined by comparison to tandemly annealed lambda DNA oligomers electrophoresed in adjacent lanes. However, the lambda size standards can only be used to measure the sizes of fragments smaller than 1200 kb since it is difficult to resolve lambda oligomers greater than 24-mers. Therefore, a complete *Not* I digest (see Table 1) of *S. pombe* chromosomal DNA was used to measure the sizes of the *Sfi* I fragments larger than 1 Mb. The size of all the *S. pombe Not* I fragments greater than 1.2 Mb had been determined physically (5).

### Ordering the *Sfi* I fragments on *S. pombe* chromosomes

About 30 genetically mapped and cloned DNA sequences were used as hybridization probes to align the *Sfi* I fragments with the genetic map and the *Not* I restriction map. For example, six telomeric fragments, **D**, **H**, **L**, **P**, **Q** and **R** were identified (Figure 2A) by hybridization to a repetitive telomeric sequence found at both ends of all three *S. pombe* chromosomes (22). The chromosomal assignment of telomeric fragments was done by purifying the three *S. pombe* chromosomal DNAs by PFG, and then digesting each with *Sfi* I and analyzing the resulting

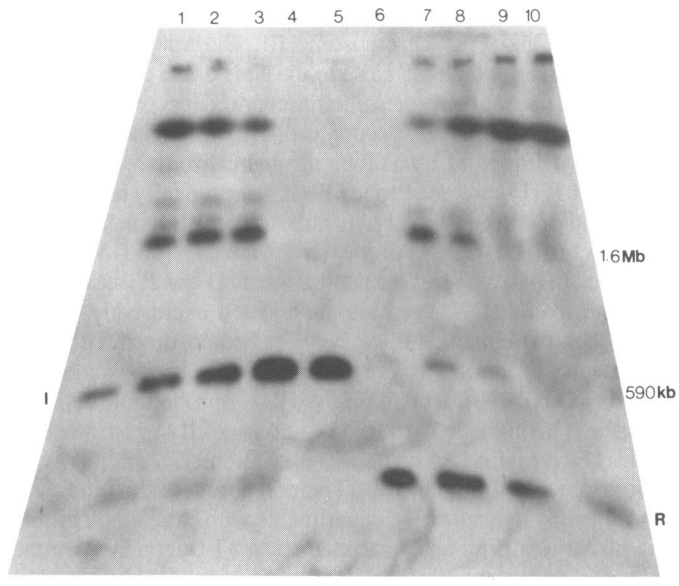



**Figure 3.** Analysis of *Not* I and *Sfi* I partial digests by hybridization to a chromosome I specific telomeric probe, pF7. PFG electrophoresis was carried out using a pulse-program: 1800 second pulse time for 60 hr at a field strength of 4.5 V/cm, 300 second pulse time for 40 hr at a field strength of 5.6 V/cm, and 100 second pulse time for 30 hr at a field strength of 6.0 V/cm. Lanes 1–5 contain *S. pombe* chromosomal DNA digested with *Not* I. The digest is progressively complete as enzyme Units: μg of DNA were 0.1:1, 0.3:1, 1:1, 3:1, and 10:1 in lanes 1–5, respectively. Lanes 6–10 contain *S. pombe* chromosomal DNA digested with *Sfi* I. The digest is progressively complete as enzyme Units: μg of DNA were 0.3:1, 1:1, 3:1, 10:1, and 30:1, in lane 10 to lane 6, respectively.

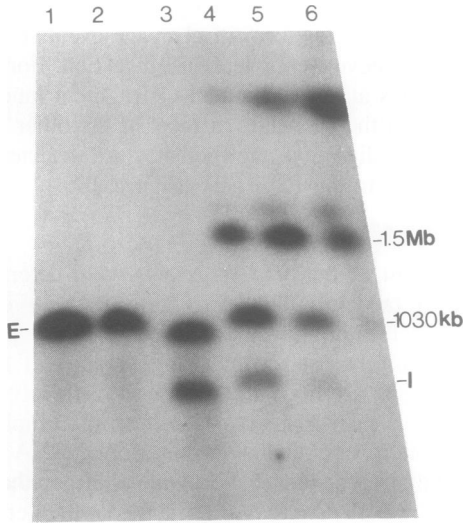



**Figure 4.** *S. pombe Not* I and *Sfi* I partial digests hybridized to a NUC2 DNA probe. PFG electrophoresis was carried out using a pulse-program: 1800 second pulse time for 60 hr at a field strength of 4.5 V/cm, 300 second pulse time for 38 hr at a field strength of 5.6 V/cm, and 100 second pulse time for 24 hr at a field strength of 6.0 V/cm. Lanes 1 and 2 contain *S. pombe* chromosomal DNA digested with *Not* I with enzyme Units: μg of DNA ratio of 10:1 and 30:1, respectively. Lane 3 contains *S. pombe* chromosomal DNA digested with both *Not* I and *Sfi* I at a ratio of enzyme Units: μg of DNA of 30:1. Lanes 4–6 contain *S. pombe* chromosomal DNA digested with *Sfi* I. The digest is progressively complete as enzyme Units: μg of DNA were 3:1, 10:1, and 30:1, in lanes 6–4, respectively.

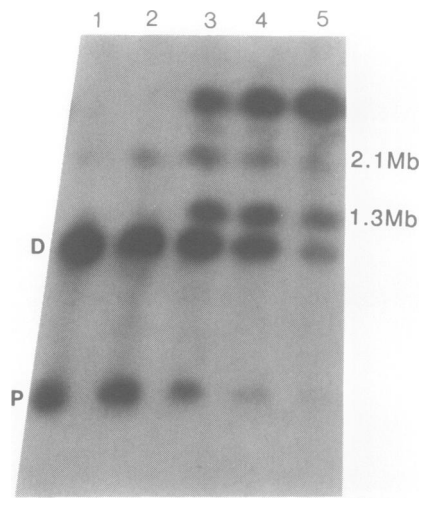



**Figure 5.** *S. pombe Sfi* I partial digests hybridized to a rDNA probe. PFG electrophoresis was carried out using a pulse-program: 3600 second pulse time for 70 hr at a field strength of 3.0 V/cm, 1800 second pulse time for 70 hr at a field strength of 3.0 V/cm, and 300 second pulse time for 75 hr at a field strength of 3.6 V/cm. From lane 5 to lane 1, the *Sfi* I digestion is progressively complete as enzyme Units: μg of DNA were 0.1:1, 0.3:1, 1:1, 5:1, and 20:1, respectively.

fragments by PFG. This located fragments **H** and **R** to chromosome I, fragments **L** and **Q** to chromosome II, and fragments **D** and **P** to chromosome III. Chromosome-specific DNA sequences, which are close to the ends of the chromosomes were used to confirm these assignment. For example, the chromosome I telomere specific probe pF7, isolated from *S. pombe Not* I-fragment **I** was found to hybridize to *S. pombe Sfi* I-fragment **R** (Figure 3). Probe CDC25, genetically assigned to the left end of chromosome I hybridized to *Sfi* I-fragment **H** (data not shown). The rDNA probe (see Materials and Methods) hybridized to two *Sfi* I fragments, fragments **D** and **P** (see below). Since the major rDNA repeats are located exclusively on the largest *Not* I fragment, i.e. *S. pombe* chromosome III (5), *Sfi* I-fragments **D** and **P** were assigned to chromosome III. Hybridization experiments with the URA4 probe indicated that fragment **D** is on the left arm of chromosome III. *Sfi* I-fragments **L** and **Q** were first identified as the telomeric fragments of chromosome II (see above). Their relative orientation was determined by hybridization experiments with αtb2 DNA probe. This probe was previously mapped to *Not* I-fragment **P** on the left arm of chromosome II (5, Figure 6). According to the *Not* I map (5), *Sfi* I-fragment **L** should contain αtb2 sequence if it were located on the left end of chromosome II. However, αtb2 hybridized to *Sfi* I-fragment **N**. Thus, *Sfi* I-fragments **L** and **Q** were assigned to the right end and left end of chromosome II, respectively. Plasmid probes, pNotH-5 and pNotH-7, isolated from *Not* I-fragment **H** hybridized to *Sfi* I-fragment **E** (data not shown).

Partial digestion strategy was also used to order some *Sfi* I fragments. For instance, the hybridization of plasmid pF7 (see Materials and Methods) to a *Sfi* I partial digest allowed the generation of a physical map spanning a region of 1.6 Mb. Plasmid pF7 hybridizes to *Not* I-fragment **I** (530 kb) and *Sfi* I-fragment **R** (110 kb) (Figure 3), respectively. The first *Not* I digestion intermediate was 1.6 Mb (lanes 1–3, Figure 3). This intermediate contains *Not* I-fragment **I** plus fragment **E** (1010 kb). The *Sfi* I digestion intermediates detected were 590 kb and 1.6 Mb (lanes 7–10, Figure 3). The 590 kb intermediate contains fragment **R** plus fragment **K** (480 kb), the 1.6 Mb intermediate contains fragment **R** plus fragments **K**, **O** (325 kb) and **I** (705 kb). The *Sfi* I digestion intermediate containing fragment **R** plus fragment **K** and **O** was not detected because *Sfi* I cleavage at the **I-O** fragment junction was very inefficient. For instance, the junction between *Sfi* I-fragments **I** and **O** was not completely cleaved even when the enzyme:DNA (Units: μg) ratio was 30:1 (lane 4, Figure 4). Instead, two *Sfi* I digestion intermediates were detected (lanes 4 and 5, Figure 4). The 1030 kb intermediate contains fragment **I** plus fragment **O**. The 1.5 Mb intermediate contains fragment **I** plus fragment **F** (770 kb). The two DNA fragments detected in lane 3 of Figure 4 are the *Not* I digestion products of the 1030 kb *Sfi* I intermediate and the *Sfi* I-fragment **I**, respectively.

The two telomeric *Sfi* I fragments (**D** and **P**) from chromosome III hybridized to the rDNA probe (lane 1, Figure 5). Additionally, two *Sfi* I digestion intermediates were detected by the rDNA probe in partially digested DNA (lanes 3–5, Figure 5). Their sizes were 1.3 Mb and 2.1 Mb, respectively. The 1.3 Mb intermediate was interpreted as fragment **D** (915 kb) plus fragment **M** (383 kb). The 2.1 Mb intermediate was interpreted as fragment P (242 kb) plus fragment B (1.9 Mb). Thus, the chromosome III *Sfi* I restriction fragment order is **D-M-B-P**. Further hybridization experiments with URA4 DNA probe confirmed this order. In addition, *Sfi* I-fragments **Q** and **S** were found to be adjacent to each other by analyses of other partial digestion data (Figure 2A).

*Not* I-*Sfi* I double digestions were performed to align some restriction sites. For example, *Not* I-*Sfi* I double digestion was used to check the order of the first four *Sfi* I fragments on the

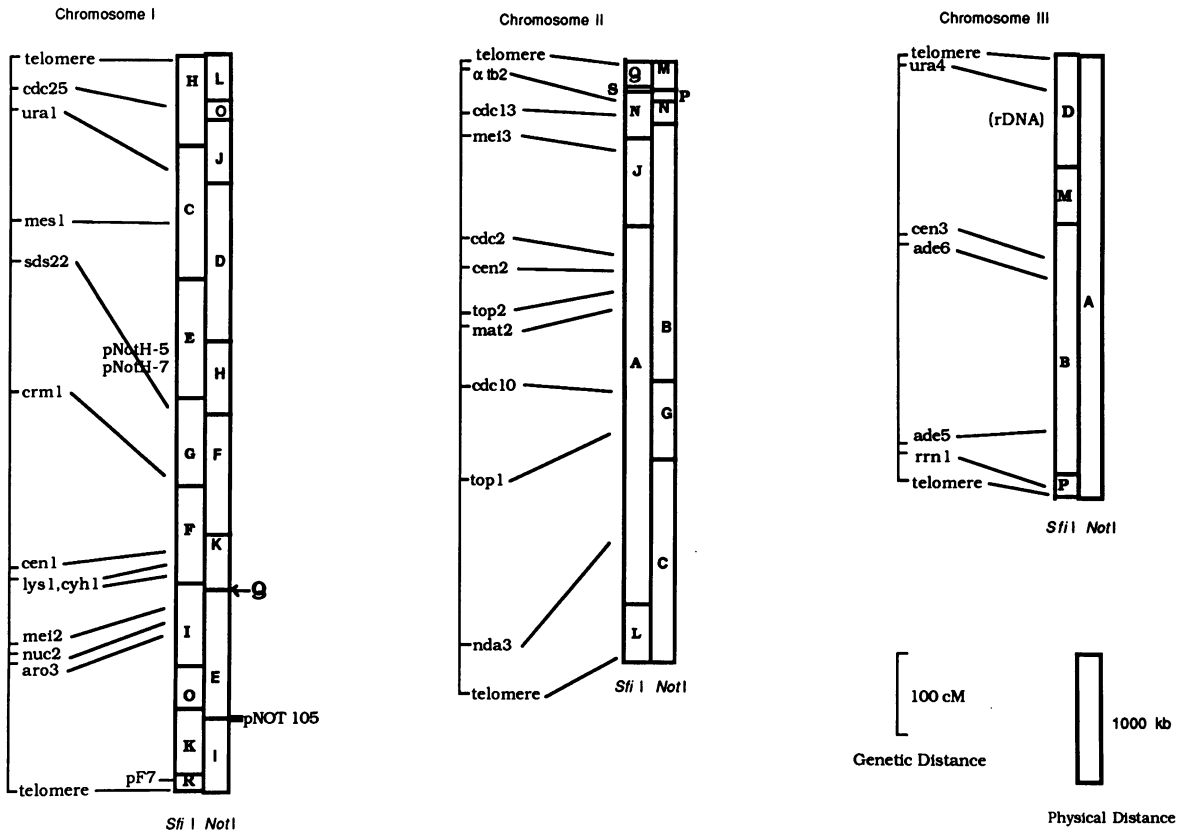



**Figure 6.** Alignment of the *Sfi* I and *Not* I restriction maps with the genetic map of the *S. pombe* genome. Genes used as hybridization probes to assign the locations of *Sfi* I and *Not* I fragment are indicated.

right arm of chromosome I. As expected, only *Sfi* I-fragments **K** and **I** were digested by *Not* I, while fragments **R** and **O** remained unchanged (data not shown). The half of *Not* I linking clone pNOT105 residing on *Not* I-fragment **E** side, at the **E-I** junction (Figure 6), was used to check the double digestion product. As expected, a 60 kb fragment was detected. Thus, the existing *Not* I restriction map aided in the construction and confirmation of the *Sfi* I physical map.

### The distribution of rDNA on *S. pombe* genome

The major (25S-5.8S-18S) rRNA genes of *S. pombe* were genetically mapped to the right arm of chromosome III (1, 23). The results shown here indicate two locations rather than one location for the rDNA, i.e. the two ends of chromosome III (Figure 5). Densitometric analyses of the autoradiograph shown in Figure 5 revealed that 73% of the rDNA repeat is located on the left arm of chromosome III (the *Sfi* I-fragment **D**). *Sfi* I-fragment **P** on the right arm only contained a small portion (27%) of rDNA. Direct scanning of the hybridized membrane on a PhosphorImager gave virtually the same result (data not shown).

## DISCUSSION

It was estimated that the *S. pombe* genome contains between 100–150 copies of the major rDNA repeat (23). Since each rDNA repeat is about 10 kb (16, 23), the *S. pombe* genome should contain at least 1000 kb of rDNA. However, the results shown here provide a different estimate of the total size of rDNA. For instance, if all the DNA content of *Sfi* I-fragment **P** (242 kb) is rDNA, then 896 kb (242 kb × 100/27 = 896 kb) of genomic DNA is rDNA in the strain studied. This is substantially smaller than the previous estimates. The multiple locations of rDNA in the *S. pombe* genome may provide a potential for homologous recombination as reported for *Escherichia coli* and *Salmonella typhimurium* (24). Crossing over between these two rDNA clusters could lead to a variety of chromosomal rearrangements. Furthermore, recent experiments have detected considerable variation in the size and distribution of rDNA in a variety of *S. pombe* strains, some of which are fairly closely related strains (J-B. Fan, C.L.Smith and C.R.Cantor, unpublished observation). It would be also very interesting to know whether both or only one of the rDNA loci are transcribed and form the nucleolar organizer.

When *Sfi* I partial digestions were performed, the first released fragments were always the telomeric fragments. For example, the first released *Sfi* I fragments are fragment **H**, from the left end of chromosome I and a digestion intermediate containing *Sfi* I-fragments **Q** and **S**, from the left end of chromosome II (lanes 3–5, Figure 2). Telomeric fragments are released first, because only a single cleavage event is needed to generate them, while two cuts are needed to generate internal fragments. In the later case, a combined telomeric fragment **Q** plus **S** is released first rather than the true telomeric fragment **Q**. This is probably due to the different reaction kinetics at different *Sfi* I restriction sites (see above).

The existing *Not* I and *Sfi* I maps allow immediate access to any segment of the genome that can be defined genetically or biochemically. The maps can be used to determine gene location. For instance, the *S. pombe* gene, CDC13 was physically localized in the experiments shown here. The physical maps can also be used to detect and characterize any chromosomal rearrangements. Indeed, with the help of the physical maps, a circular chromosome II was found in one *S. pombe* strain (M. Rochet, J-B. Fan and C.L. Smith, unpublished data.). Polymorphisms have also been detected in chromosome I (J-B. Fan and C.L.Smith, unpublished observations). Comparison of the physical maps with the genetic maps allows the identification of genetic recombinational hot spots and cold spots (5).

These maps, and the DNA fragments, are being used to order a genomic library in a top-down mapping approach (D. Grothues, C.R. Cantor and C.L. Smith, unpublished data). Here, hybridization probes from specific large restriction fragments are used to regionally assign clones, which are further ordered by various fingerprinting approaches. This approach should considerably speed chromosome library ordering.

## ACKNOWLEDGEMENT

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## REFERENCES

1. Kohli, J. (1987) *Current Genetics* **11**, 575–589.
2. Moreno, S., Klar, A. and Nurse, P. (1991) *Methods in Enzymology* **194**, 795–823.
3. Allshire, R.C., Cranston, G., Gosden, J.R., Maule, J.C., Hastie, N.D. and P. A. Fantes, P.A. (1987) *Cell* **50**, 391–403.
4. Chikashige, Y., Kinoshita, N., Nakaseko, Y., Matsumoto, T., Murakami, S., Niwa, O. and Yanagida, M. (1989) *Cell* **57**, 739–751.
5. Fan, J-B., Chikashige, Y., Smith, C.L., Niwa, O., Yanagida, M. and Cantor, C.R. (1989) *Nucleic Acids Research* **17**, 2801–2818.
6. Hahnenberger, K.M., Baum, M.P., Polizzi, C.M., Carbon, J. and Clarke, L. (1989) *Proc. Natl. Acad. Sci.* USA **86**, 577–581.
7. Russell, P. (1989) In Nasim, A., Young, P. and Johnson, B.F. (eds.), Molecular Biology of the Fission Yeast, Academic Press, San Diego. pp. 243–271.
8. Smith, C.L., Matsumoto, T., Niwa, O., Klco, S., Fan, J-B., Yanagida, M. and Cantor, C.R. (1987) *Nucleic Acids Research* **15**, 4481–4489.
9. Booher, B. and Beach, D. (1989) *Cell* **57**, 1009–1016.
10. Fantes, P. (1989) In Nasim, A., Young, P. and Johnson, B.F. (eds.), Molecular Biology of the Fission Yeast, Academic Press, San Diego, pp. 127–204.
11. Kinoshita, N., Ohkura, H. and Yanagida, M. (1990) *Cell* **63**, 405–415.
12. Moreno, S., Hayles, J. and Nurse, P. (1989) *Cell* **58**, 361–372.
13. Uemura, T., Ohkura, H., Adachi, Y., Morino, K., Shiozaki, K. and Yanagida, M. (1987) *Cell* **50**, 917–925.
14. Schwartz, D.C. and Cantor, C.R. (1984) *Cell* **37**, 67–75.
15. Gutz, H., Heslot, H., Leupold, U. and Loprieno, N. (1974) In King, R.C. (ed.), Handbook of Genetics, Plenum Press, New York, pp. 395–446.
16. Schaak, J., Mao, J-i. and Soll, D. (1982) *Nucleic Acids Research* **10**, 2851–2864.
17. Mathew, M.K., Smith, C.L. and Cantor, C.R. (1988) *Biochemistry* **27**, 9204–9210.
18. Smith, C.L., Klco, S. and Cantor, C.R. (1988) In Davis, K. (ed.), Genome analysis: A Practical Approach, IRL Press, Oxford, England, pp. 41–72.
19. Fang, H., Zhang, T-Y., Oliva, R., Fan, J-B., Nikolic, J. and Smith, C.L. (1991) In Naylor, S. (ed.), Mapping the Human Genome: Methodology and the Application to Human Disease, Academic Press, San Diego, in Press.
20. Feinberg, A.O. and Vogelstern, B. (1984) *Analytical Biochemistry* **137**, 266–267.
21. Condemine, G. and Smith, C.L. (1990) In Drlica, K. and Riley, M. (eds.), The Bacterial Chromosome, American Society for Microbiology, Washington, D.C. pp. 53–60.
22. Sugawara, N. and Szostak, J.W. (1986) *Yeast* **2** (supp), s373.
23. Toda, T., Nakaseko, Y., Niwa, O. and Yanagida, M. (1984) *Current Genetics* **8**, 93–97.
24. Hill, C.W., Harvey, S. and Gray, J.A. (1990) In Drlica, K. and Riley, M. (eds.), The Bacterial Chromosome, American Society for Microbiology, Washington, D.C. pp. 335–340.