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Detection and characterization of a ring chromosome in the fission yeast *Schizosaccharomyces pombe*

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ABSTRACT

***Not*I and *Sfi*I genomic restriction maps were used to detect and characterize a ring chromosome II in a *Schizosaccharomyces pombe* strain with a meitotic defect on chromosome II. The ring chromosome was formed by an intrachromosomal fusion near, or at, the very ends of chromosome II.**

INTRODUCTION

The fission yeast *Schizosaccharomyces pombe* is a well characterized single-celled eukaryote (1). More than 460 genes have been defined by mutation analysis and by molecular cloning (1, 2). Over 270 genes have been genetically mapped either to one of the three linkage groups (which make up a total meiotic map length of 1700 centimorgans (cM)) or to the mitochondrial genome (1, 2, 3). The three chromosomes from *S.pombe* can be visualized microscopically (4) and have been resolved by pulsed field gel electrophoresis (PFG: 5). The sizes of the chromosomes are 5.7 megabase pairs (Mb), 4.7 Mb, and 3.5 Mb for chromosome I, II, and III, respectively, as determined from the *Not*I and *Sfi*I restriction maps (6, 7). The existing physical maps should allow immediate access to any segment of the genome that can be detected genetically or biochemically. They can also be used to indicate the occurrence of large DNA rearrangements on the genome. This work reports the detection and characterization of a ring chromosome II in *S.pombe*.

MATERIALS AND METHODS

Wild type *S.pombe* strain, 971 h^- (8) (obtained from M.Yanagida) was used in this study. Strains MR102 (h^+ *dea2 ada1-102*) and MR414 (h^+ *dea2 ada1-414*) were obtained from the authors' collection. Strain MR414 and MR102 were derived from strains isolated in a screen for adenosine deaminase mutants starting with an h^+*ade10-1 dea2* strain (Rochet *et al.*, unpublished results). The *ada1* locus probably encodes an adenosine deaminase (Rochet *et al.*, unpublished results). Growth media and genetic methods were those described by Gutz *et al.* (8), except that the minimal media contained 750 mg/L, instead of 5 mg/L, $(NH_4)_2SO_4$. Protocols for the purification of yeast chromosomal DNA in agarose inserts and subsequent enzyme digestion, as well as PFG fractionation conditions were described previously (6, 9).

RESULTS AND DISCUSSION

Circular form of chromosome II detected in strain MR414 by PFG

Intact *S.pombe* chromosomal DNAs made in agarose blocks were fractionated by PFG (Figure 1). As shown in Figure 1, three chromosomal DNA bands were detected for *S.pombe* strains *972* h^- (lane 1) and MR102 (lane 2), while only two chromosomal bands (chromosome I and III) were detected for strain MR414 (lane 3). It appeared that chromosome II is missing from the latter strain. However, it is not likely that *S.pombe* cells could survive missing the entire chromosome II. Instead, the results suggested that chromosome II in strain MR414 might have some special DNA conformation that caused abnormal electrophoretic behavior. For instance, experiments (12) with the 4.7 Mb (13, 14) circular *Escherichia coli* chromosome predict that a circular 4.7 Mb *S.pombe* chromosome II would not enter an agarose gel under the PFG fractionation conditions shown in Figure 1. The recombination frequency among genetic markers, as well as the observed inhibition of meiotic recombination on chromosome II of strain MR414 were consistent with the circular model (Rochet *et al.*, unpublished observations). For instance, at least two cross-overs are required for one successful recombinational event to occur between two circular chromosomes or between one circular and one linear chromosome.

An attempt was made to use γ-irradiation to prove the existence of a ring chromosome II in strain MR414. Genomic DNA from

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strain MR414 was subjected to ^{137}Cs γ-radiation before PFG in order to linearize any putative circular molecules. In these experiments, the irradiated sample had sub-stoichiometric amounts of a DNA species at the expected PFG position for chromosome II. This result was not surprising. Megabase DNA is exquisitely sensitive to nuclease contamination. Furthermore, Poisson statistics predicts that the maximum number of molecules that can exist as intact linear forms containing single, double-strand breaks, is one third.

Conversion of two telomeric *Not*I fragments from chromosome II to one larger fragment in strain MR414

The *Not*I (6) and *Sfi*I (7) restriction maps of the *S.pombe* genome were used to characterize the genomic rearrangements in strain MR414 directly. Intact genomic DNAs made from strains MR414, MR102 and 972 h^- were digested with restriction enzyme *Not*I and fractionated by PFG (Figure 2A and B). All the *Not*I fragments from chromosome I and III, and all the internal *Not*I fragments from chromosome II from strain MR414 were exactly the same as those in strains 972 h^- and MR102. However, the two telomeric *Not*I fragments of chromosome II, fragments C (Figure 2A lane 3) and M (Figure 2B lane 2) were missing from strain MR414. Instead, a new fragment, called fragment X was found (Figure 2A lane 3). Fragment X was 1.8 Mb in size. This size corresponds to the combined sizes of fragment C (1.5 Mb) plus fragment M (0.24 Mb). This result suggested that strain MR414 contains an intrachromosomal fusion near or at the very ends of the chromosome II and that the fusion chromosome retained almost all the DNA content of chromosome II. Analysis of the *Sfi*I restriction fragment pattern of strain MR414 further supported this interpretation (data not shown).

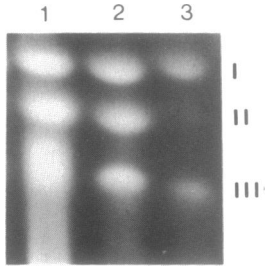

Figure 1. Intact *S.pombe* chromosomal DNAs fractionated by PFG. PFG analysis was carried out on an LKB Pulsaphor apparatus at 3 V/cm for 150 hr using a 4500 sec pulse time. Lanes 1–3 contain chromosomal DNAs from *S.pombe* strains 972 h^-, MR102 and MR414, respectively.

Loss of telomeric sequences from the ring chromosome II

Strong hybridization signals were obtained with the *S.pombe* telomeric sequence probe, contained on plasmid pSPT16 for the *Not*I telomeric fragments of chromosome I from (*Not*I fragment L and I) and chromosome III (*Not*I fragment A) from strain MR414. However, this same probe gave a very weak hybridization band for *Not*I fragment X, (Figure 2C lane 3). Densitometric analysis of the autoradiograph shown in Figure 2C indicated that the ratio of the hybridization signal of *Not*I fragment A (3.5 Mb) to that of *Not*I fragment C (1.5 Mb) for strain MR102 was 2.5:1 (data not shown). If the *Not*I fragment strain MR414 retained all the telomeric repeat sequences from *Not*I fragment C and *Not*I fragment M, the ratio of hybridization signal of *Not*I fragment A to that of *Not*I fragment X (Figure 2C lane 3) should be smaller than 2.5:1. However, densitometric analysis gives a significantly larger ratio of 4:1 (data not shown). This suggests that many, if not all, of the telomeric repetitive sequences on chromosome II were lost during the formation of the ring chromosome. Alternatively, interstitial telomeric sequences in a ring chromosome may have been lost by recombination within the repeat element after fusion. It is noteworthy that the ratio of the hybridization signals of *Not*I

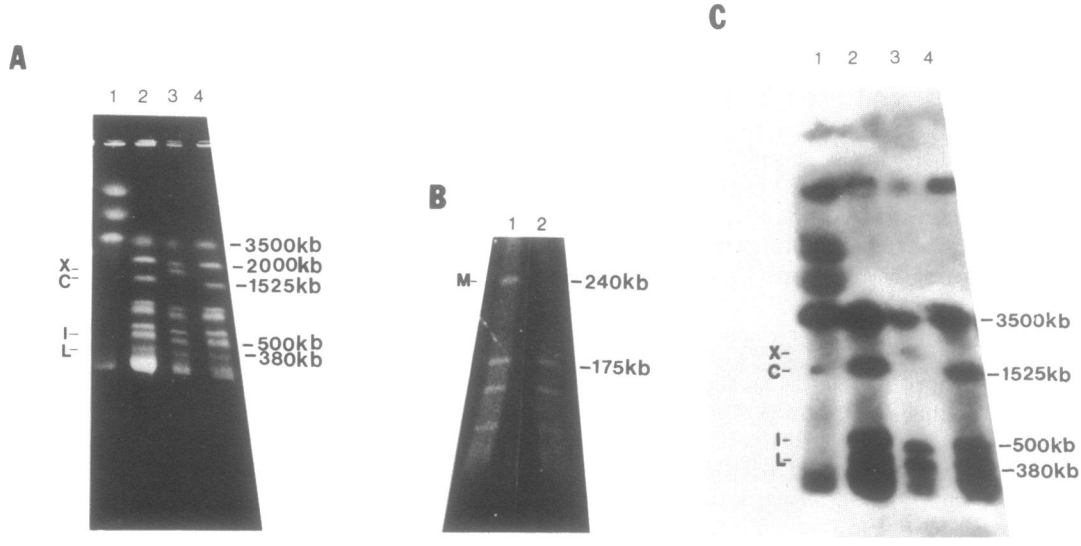

Figure 2. PFG fractionation and hybridization analysis of *S.pombe* chromosomal DNAs digested with the restriction enzyme *Not*I. (**A**) PFG analysis was carried out using a pulse-program: 4500 sec pulse time for 100 hr; 800 sec pulse time for 46 hr and 200 sec pulse time for 22 hr at a field strength of 3 V/cm. Lane 1 contains intact chromosomal DNAs from strain 972 h^-. Lanes 2–4 contain *Not*I digests of DNAs from strain MR102, MR414 and 972 h^-, respectively. (**B**) PFG analysis was carried out at 10 V/cm for 30 hr using a 20 sec pulse time. Lane 1 contains strain MR102 DNA digested with *Not*I. Lane 2 contains strain MR414 DNA digested with *Not*I. (**C**) Hybridization of the PFG gel shown in (A) with *S.pombe* telomeric probe pSPT16.

fragments A and I (500 kb) remained constant in lanes 2, 3 and 4 of Figure 2C.

A circular chromosome generated by homologous recombination in the budding yeast *Saccharomyces cerevisiae* and was used to study interhomolog recombination and sister-chromated exchange in yeast (15, 16). In *Drosophila melanogaster*, a ring chromosome was found to be composed of two X chromosomes joined at both ends (17). The circular *S.pombe* chromosome II could have been formed in a number of ways. Clarification of how the circular chromosome II was formed awaits further characterization of the junction point. However, the loss of most of the telomeric repeats from the fusion *Not*I fragment suggests that homologous recombination between the telomeric repeats might be responsible for generating the ring chromosome.

The occurrence of a large ring chromosome in *S.pombe* provides a means to understand more about the behavior and formation of aberrant chromosomal structures. For example, how did the circularization of the chromosome arise? Is there any equilibrium between the circular and the linear forms of the chromosome? The ring chromosome can also be used as unique material to study basic biological problems, such as DNA replication, DNA recombination, gene transcription, chromosome dynamics and chromosome segregation (18). For instance, preliminary experiments indicate that diploids heterozygous for ring chromosome II are fairly unstable (data not shown).

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REFERENCES

1. Moreno,S., Klar,A. and Nurse,P. (1991) *Methods Enzymol.* **194**, 795–823.
2. Kohli,J. (1987) *Curr. Genet.* **11**, 575–589.
3. Gygax,A. and Thuriaux,P. (1984) *Curr. Genet.* **8**, 85–92.
4. Robinow,C.F. (1977) *Genetics* **87**, 491–497.
5. Smith,C.L., Matsumoto,T., Niwa,O., Klco,S., Fan,J.-B., Yanagida,M. and Cantor,C.R. (1987) *Nucleic Acids Res.* **15**, 44814489.
6. Fan,J.-B., Chikashige,Y., Smith,C.L., Niwa,O., Yanagida,M. and Cantor,C.R. (1989) *Nucleic Acids Res.* **17**, 2801–2818.
7. Fan,J.-B., Grothues,D. and Smith,C.L. (1991) *Nucleic Acids Res.* **19**, 6289–6294.
8. Gutz,H., Heslot,H., Leopold,U. and Loprieno,N. (1974) In King,R.C. (ed.), *Handbook of Genetics*. Plenum Press, New York, pp. 395–446.
9. Smith,C.L., Klco,S. and Cantor,C.R. (1988) In Davis,K. (ed.), *Genome Analysis: A Practical Approach*. IRL Press, Oxford, UK, pp. 41–72.
10. Allshire,R.C., Gosden,J.R., Cross,S.H., Cranston,G., Rout,D., Sugawara,N., Szostak,J.W., Fantes,P.A. and Hastie,N.D. (1988) *Nature* **332**, 656–659.
11. Sugawara,N. and Szostak,J.W. (1986) *Yeast*, **2** (suppl.), s373.
12. Smith,C.L. and Condemine,G. (1990) *J. Bact.* **172**, 1167–1172.
13. Smith,C.L., Econome,J.G., Schutt,A., Klco,S. and Cantor,C.R. (1987) *Science* **236**, 1448–1453.
14. Kohara,Y., Akiyama,K. and Isono,K. (1987) *Cell* **50**, 495–508.
15. Klar,A.J.S., Strathern,J.N., Hicks,J.B. and Prudente,D. (1983) *Mol. Cell. Biol.* **3**, 803–810.
16. Game,J.C., Sitney,K.C., Cook,V.E. and Mortimer,R.K. (1989) *Genetics* **123**, 695–713.
17. Traverse,K.L. and Pardue,M.L. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8116–8120.
18. Murray,A.W. and Szostak,J.W. (1985) *Annu. Rev. Cell Biol.* **1**, 289–315.