Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4043–4047, June 1990
Genetics

Introduction of large linear minichromosomes into *Schizosaccharomyces pombe* by an improved transformation procedure

(Lipofectin/yeast artificial chromosome/*Saccharomyces cerevisiae*/genome mapping/telomeres)

ROBIN C. ALLSHIRE

Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724

Communicated by James D. Watson, March 5, 1990

ABSTRACT **The efficiency of transformation of *Schizosaccharomyces pombe* has been increased 10- to 50-fold over previously reported methods. By using 1 μg of plasmid, 7.0 × 10^5 transformants are regularly obtained. This increased transformation efficiency is mainly due to the inclusion of the cationic liposome-forming reagent Lipofectin in the protocol. Various parameters affecting transformation of *Sc. pombe* in the presence of Lipofectin have been examined. Lipofectin can also be used to increase transformation efficiency in *Saccharomyces cerevisiae*. It is also demonstrated that by using this improved transformation procedure, linear minichromosomes of >500 kilobases can be introduced into *Sc. pombe* with relative ease. These minichromosomes can replicate as stable linear molecules upon reintroduction into *Sc. pombe*, demonstrating that *Sc. pombe* telomeres retain function when reintroduced as naked DNA. The ability of *Sc. pombe* to admit large DNA molecules indicates that it should be feasible to clone large DNA from other organisms in *Sc. pombe*.**

The identification of the elements required to construct a minichromosome functional in *Saccharomyces cerevisiae* (1) has led to the development of the yeast artificial chromosome (YAC) cloning system (2). The YAC system allows large DNA of up to 1000 kilobases (kb) from any organism to be propagated in *S. cerevisiae* as an additional chromosome (2–5). Genomic libraries can be constructed with an average insert size of 220–410 kb (6, 7). This provides a 10-fold increase in the cloning capacity of YACs over any previously described cloning system.

The fission yeast, *Schizosaccharomyces pombe*, provides a well-studied alternative to *S. cerevisiae*. The chromosomes of *Sc. pombe* are all longer than those of *S. cerevisiae* (8, 9). Therefore, *Sc. pombe* has an innate ability to deal with DNA of >2000 kb. It is plausible that a cloning system in *Sc. pombe* might allow the so called "cytogenetic-cloning gap" to be bridged. In addition, it has previously been demonstrated that intact *Sc. pombe* chromosomes can be introduced into mammalian cells (10). If large fragments of DNA can be introduced into *Sc. pombe*, then this provides a powerful shuttle system for identifying and characterizing genes and chromosomal regions of interest.

A major factor contributing to the development of YAC cloning was the ability to obtain a high transformation frequency (>1 × 10^6 per μg of plasmid per 3 × 10^7 protoplasts; refs. 2 and 11). The efficiency of *Sc. pombe* transformation remains low—2–3 × 10^4 transformants per μg of plasmid per 3–5 × 10^7 protoplasts (12, 13). A first step in the development of a system allowing large DNA to be cloned in *Sc. pombe* is to improve the transformation efficiency. Here, the effect of the reagent Lipofectin on *Sc. pombe* transformation is investigated. The protocol for transformation of *Sc. pombe* is optimized so that efficiencies of 7 × 10^5 transformants per μg of plasmid per 4 × 10^7 protoplasts are regularly obtained. By using this method, it is demonstrated that large DNA molecules (>500 kb) can be introduced into *Sc. pombe*.

MATERIALS AND METHODS

Strains. For the studies described here, the *Sc. pombe* strain Sp813 (*h^{+N} leu1-32 ura4-D18 ade6-210*) was used in all transformation experiments. The minichromosome strain HM348-23R (*h^+ leu1 fur1 tps16-112 ade6-210* Ch16-23R) was provided by M. Yanagida and is described in ref. 14. The *S. cerevisiae* strain AB1380 (ref. 2; *MATα Ψ^+ ura3 trp1 ade2-1 can1-100 lys2-1 his5*) was used. Standard *Sc. pombe* media (YE, YEA, PM, and PMA) have been described (15).

Plasmids. The plasmids pIRT2 (16) and pIRT/URA4 (similar to pIRT2 except that the 2.18-kb *Hin*dIII fragment, which carries the *S. cerevisiae Leu2* gene in pIRT2, is replaced with a 1.4-kb *Hin*dIII fragment bearing the *Sc. pombe Ura4* gene) were used in this study. YCP24, containing the *S. cerevisiae URA3* gene, was used in *S. cerevisiae* transformations.

***Sc. pombe* Transformation.** The transformation method is based on that described by Beach *et al.* (13). Between 0.5 and 4 ml of a fresh overnight culture of cells grown in a rich medium such as YEA (0.5% yeast extract/3% glucose/75 mg of adenine per ml) was used to inoculate four 100-ml quantities of minimal medium (PM) supplemented with 75 mg of amino acids per ml and 0.5% glucose with four different-sized inocula (e.g., 0.5, 1.0, 1.5, and 2.0 ml of overnight culture).

Each 100-ml culture was harvested and the cells were resuspended separately in a total of 20 ml of SP 1 (1.2 M sorbitol/50 mM sodium citrate/50 mM sodium phosphate/40 mM EDTA, pH 5.6), 20 μl of 2-mercaptoethanol was then added and the cells were incubated at 25°C for 10 min. All four tubes of cells were centrifuged and each pellet was resuspended in 10 ml of SP 2 (1.2 M sorbitol/50 mM sodium citrate/50 mM sodium phosphate, pH 5.6), and 20 mg of Novozym 234 (Novo Biolabs) was added to each tube, incubated at 37°C, and monitored every 10 min for the formation of spherical protoplasts. In general, cultures with a starting density of 1.5–2.5 × 10^7 cells per ml form protoplasts most efficiently; however, this is not always predictable.

On obtaining between 70% and 90% protoplasts with one or more of the starting cultures, 30 ml of SP 3 (1.2 M sorbitol/10 mM Tris·HCl, pH 7.6) was added and gently mixed. The protoplasts were harvested and washed twice with 30 ml of SP 3. After the final wash, the protoplasts were resuspended gently in 1 ml of SP 4 [1.2 M sorbitol/10 mM Tris·HCl/10 mM $CaCl_2 \cdot 2H_2O$, pH 7.6 (STC)] and 100-μl

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: YAC, yeast artificial chromosome; CHEF, contour-clamped homogeneous electric field.

aliquots of protoplasts in SP 4 were mixed with plasmid DNA in the presence or absence of 1 μg of carrier DNA (sonicated calf thymus DNA). Protoplasts were diluted in SP 4 to test the effect of protoplast concentration on transformation. After 15 min of incubation at room temperature, an equal volume of SP 4 with or without Lipofectin (BRL) was added and the protoplast/DNA mixture was incubated for an additional 15 min. Finally, 1 ml of SP 5 (20% polyethylene glycol 3500–4000/10 mM Tris·HCl, pH 7.6/10 mM $CaCl_2 \cdot 2H_2O$) was added and mixed with the protoplasts by gently inverting the tube. After a 15-min incubation at room temperature, the protoplasts were recovered by centrifugation at 600 × *g*. The pellet was resuspended in 200–2000 μl of SP 4 and various aliquots were spread directly onto minimal medium plates (PM) containing 1.2 M sorbitol and were supplemented with required amino acids at 75 mg/ml. Plasmid transformants were visible after 4 days incubation at 32°C, but plates were not counted until 7 or 8 days posttransformation.

***S. cerevisiae* Transformations.** Spheroplasts of *S. cerevisiae* strain AB1380 were prepared by digestion with lyticase (Sigma). The transformation procedure was essentially as described (11) except for the inclusion of an additional 15-min incubation in STC with or without Lipofectin at 33 μg/ml prior to the addition of polyethylene glycol. Transformants were selected on synthetic dextrose plates lacking uracil.

Preparation of *Sc. pombe* Chromosome-Sized DNA and Pulsed-Field Gel Electrophoresis. The procedure for preparing chromosome-sized *Sc. pombe* DNA was essentially as described by Niwa *et al.* (17). The minichromosome Ch16 was separated by electrophoresis on a "homemade" contour-clamped homogeneous electric field (CHEF) apparatus (8). The samples were run on 1% agarose gels in 0.5× TBE at 200 V with an initial pulse time of 60 sec, which, after 14 hr, was changed to 90 sec and the gel was run for an additional 10 hr. The buffer was cooled to 12°C during electrophoresis.

To prepare minichromosomes for transformation, the chromosomes were run under the same conditions but on a 1% low melting point agarose gel (BRL). The agarose containing the minichromosome to be used in subsequent transformations was not stained with ethidium bromide. Only flanking tracks were stained; the position of the minichromosome in these tracks was marked by nicking with a scalpel, the gel was reassembled, and the agarose containing unstained minichromosome was cut out and placed in 50 mM NaCl/10 mM Tris·HCl, pH 8.0/5 mM EDTA and stored at 4°C.

Transformation with Gel-Purified Minichromosomes. Prior to transformation, a portion of agarose (50-μl slice) containing the minichromosome was removed and dialyzed against 10 vol of 10 mM Tris·HCl/1 mM EDTA, pH 8.0 (TE), at room temperature for 30 min. This was repeated twice. The agarose slice containing the minichromosome was then mixed with a slice (10 μl) of agarose containing high molecular weight mammalian DNA (this protects the minichromosome from shearing in subsequent steps). All excess TE was removed from the agarose slices. The agarose was then melted at 65°C for 5 min, cooled to 37°C, and 10-μl aliquots were used to transform *Sc. pombe* protoplasts in the presence of 1 μg of carrier DNA. The molten agarose was pipetted by using a cut off Pipetman tip. Minichromosome transformants are only visible on plates after 7 days at 32°C. The recipient strain SP813 carries the *ade6-210* auxotrophic allele. When grown on YE plates, colonies develop a dark red color. The *ade6-216* auxotrophic allele develops a pink color on YE plates. When both alleles (*-210* and *-216*) are present in a cell, intragenic complementation occurs resulting in white, Ade^+, prototrophic colonies. The minichromosome Ch16-23R carries the *LEU2* marker and the *ade6-216* allele. Minichromosome transformants were initially selected on minimal plates lacking leucine for the presence of *LEU2*. Subsequently, these Leu^+ transformants were tested for adenine prototrophy by screening for colony color and by their ability to grow on minimal plates lacking adenine.

Southern Transfer and Hybridization. Southern blots of pulsed-field gels were prepared by standard methods. The plasmid pucURA4 (10) was labeled with [α-^{32}P]ATP by random-primer synthesis (18). Hybridizations were performed as described (19) and filters were washed several times in 2× SSC at 68°C (1× SSC = 0.15 M NaCl/0.015 M sodium citrate).

RESULTS

Lipofectin Enhances Transformation of Both *Sc. pombe* and *S. cerevisiae*. Recently, a transformation procedure for mammalian cells was described that used the positively charged liposome-forming molecule *N*-[1-(2,3-dioleyloxy)propyl]-*N,N,N*-trimethylammonium chloride (20, 21). These liposomes form complexes with DNA and cell membranes, giving high-frequency transformation. It seemed reasonable to assume that Lipofectin might interact in a similar fashion with the cellular membranes of *Sc. pombe* and *S. cerevisiae* to facilitate the uptake of DNA.

The optimal concentration of Lipofectin used for transformation of mammalian cells has been found to be between 30 and 50 μg/ml (21). In Fig. 1, the frequency of *Sc. pombe* transformation with 1 μg of a 6.0-kb plasmid that carries the *Sc. pombe Ura4* gene (pIRT/URA4) is plotted against the concentration of Lipofectin. Lipofectin was added to standard *Sc. pombe* transformations for 15 min after a 15-min incubation with DNA. At a final Lipofectin concentration of 33 μg/ml, the number of transformants peaked at 7 × 10^5 transformants per μg, which is 35 times greater than a standard *Sc. pombe* transformation (0 μg of Lipofectin per ml; Fig. 1). Fig. 2*A* compares plates resulting from a standard and a Lipofectin-enhanced transformation. Lipofectin can also enhance transformation of *S. cerevisiae* spheroplasts (Fig. 2*B*). However, this is not investigated further here.

Transformation in the Presence of Carrier Is Linear with Increasing Plasmid Concentration. In the above experiments, 1 μg of plasmid was used throughout. This quantity of

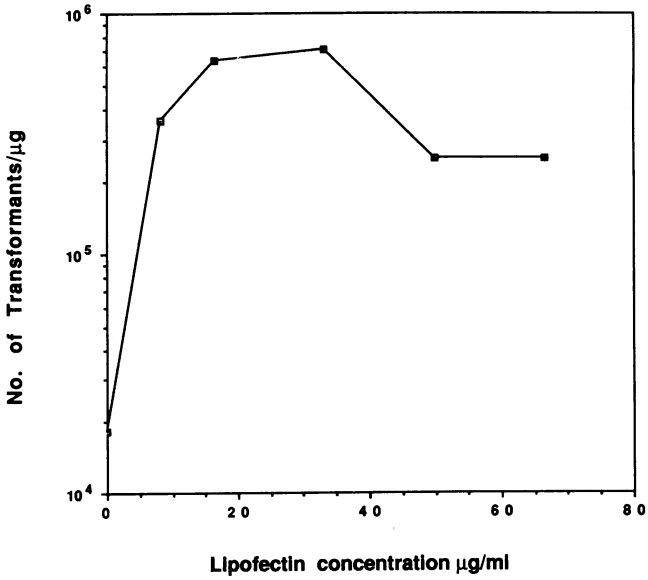

FIG. 1. Effect of increasing concentration of Lipofectin on *Sc. pombe* transformation efficiency. pIRT/URA4 (1 μg) was incubated with SP813 protoplasts (100 μl) at a concentration of ≈5 × 10^8 per ml for 15 min followed by the addition of 100 μl of SP 4 containing 0–66 μg of Lipofectin per ml. All points plotted represent a mean from duplicate transformations.

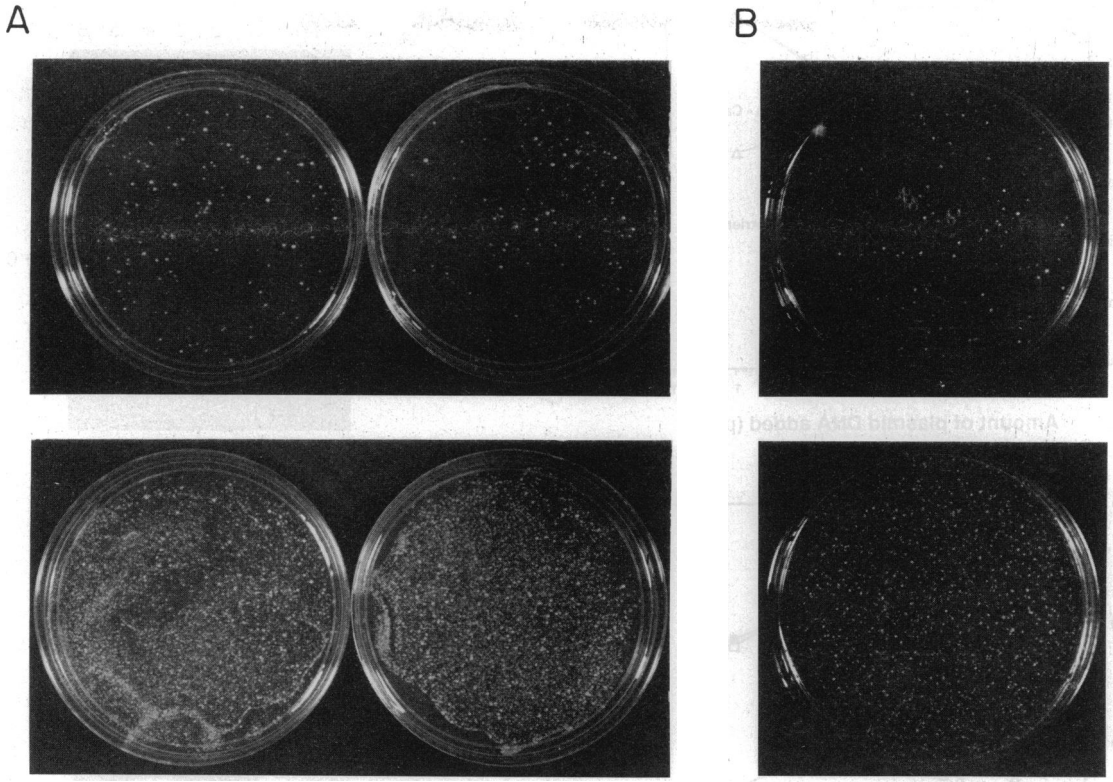

FIG. 2. (*A*) Photograph of plates resulting from the transformation of *Sc. pombe* SP813 with 1 μg of pIRT/URA4 in the absence (*Upper*) or presence (*Lower*) of Lipofectin at a final concentration of 33 μg/ml. These plates resulted from the experiment shown in Fig. 1. Each plate was seeded with 1/100th of each transformation. (*B*) Photograph of plates resulting from the transformation of *S. cerevisiae* AB1380 with 1 μg of YCP24 in the absence (*Upper*) or presence (*Lower*) of Lipofectin at a final concentration of 33 μg/ml. Plates seeded with 1/10th of each transformation are shown.

plasmid in a 100-μl transformation mixture containing ≈5 × 10^7 protoplasts may well be saturating. To investigate this, the amount of plasmid pIRT/URA4 was titrated. This should reveal whether transformation of *Sc. pombe* is linear with respect to increasing DNA concentration and determine the concentration of plasmid at which maximum transformation frequency is obtained in the presence of 33 μg of Lipofectin per ml. The transformations were performed in the presence and absence of carrier DNA since it had been reported previously that carrier DNA facilitates transformation with low concentrations of plasmid (13). For comparison, a plasmid titration was also performed in the absence of Lipofectin. Fig. 3 shows that the increase in the absolute number of transformants obtained with increasing amounts of plasmid is linear only in the presence of carrier DNA (1 μg per 100 μl of protoplasts). In the absence of carrier DNA, there is a sharp increase in the number of transformants obtained at higher DNA concentrations (>100 ng per transformation) where the plasmid appears to act as its own carrier. In Fig. 3*B* the same data are plotted as extrapolated transformants per μg against the amount of plasmid added. This shows that transformation frequencies of >1 × 10^6 per μg can be obtained at low plasmid concentrations (1–100 ng per 100 μl of protoplasts) when performed in the presence of carrier DNA and Lipofectin. It is clear that when >100 ng of plasmid is used in a transformation, saturation is reached and transformation frequency declines.

The optimum number of protoplasts required in a *Sc. pombe* Lipofectin-enhanced transformation to give most efficient transformation was also investigated. A batch of SP813 protoplasts (5 × 10^8 viable cells per ml) was serially diluted and aliquots of each dilution were transformed with 10 ng of pIRT/URA4 in the presence of 1 μg of carrier DNA followed by a 15-min incubation with 33 μg of Lipofectin per ml.

In transformations containing 10 ng of plasmid and 100 μl of cells, maximum transformation efficiencies (6–8 × 10^5 per μg) were obtained at protoplast concentrations of between 1 and 5 × 10^8 per ml. Reasonable transformation efficiencies of 6 × 10^4 per μg can still be obtained when only 1.5 × 10^6 protoplasts are present in a transformation (data not shown). Therefore, where transformation efficiency is not critical, many transformations (≈300) could be performed on a single batch of protoplasts resulting from a 100-ml starting culture.

Transformation of *Sc. pombe* with Large Linear DNA. The enhancement of *Sc. pombe* transformation with Lipofectin described above may allow efficient uptake of large DNA molecules. Niwa *et al.* (14, 17) described the construction of minichromosome derivatives of *Sc. pombe* chromosome III by irradiation. The 550-kb minichromosome Ch16 in the strain HM348-23R (14) was used to test transformation of *Sc. pombe* SP813 with large DNA. This minichromosome carries the *S. cerevisiae Leu2* gene integrated close to the *fur1* locus, and the *ade6-216* (pink) allele (see *Materials and Methods*; ref. 21). The successful introduction of Ch16 into SP813 should result in white Ade^+ Leu^+ colonies by complementation of *ade6-210* (red) and *leu1-32*. Since the *ura4* locus is deleted in SP813 (*ura4-D18*), real Ch16 transformants can be distinguished from the donor strain HM348-23R, which is $Ura4^+$. This distinction can be made both genetically and by hybridization with the *ura4* gene.

The Ch16 minichromosome was prepared and introduced into SP813 as described above. From four independent transformations, performed in the presence of Lipofectin, 324 Leu^+ colonies were obtained. Only 10 μl of molten agarose, estimated to contain between 1 and 10 ng of the minichromosome, was added to each transformation mix-

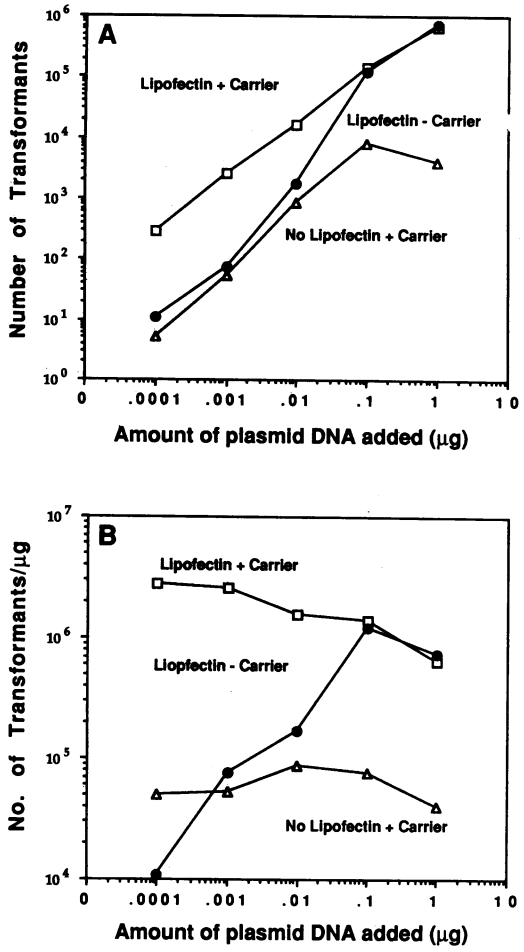

FIG. 3. Titration of added plasmid DNA in Lipofectin-enhanced or normal *Sc. pombe* transformation in the presence and absence of carrier DNA. (*A*) Absolute number of transformants resulting from transformations performed with increasing amounts of added pIRT/URA4. Titrations were performed in the presence of carrier DNA with no Lipofectin (△) and in the presence of Lipofectin with (□) or without (●) carrier DNA. Protoplast concentration was ≈5 × 10^8 per ml. Carrier DNA was added with the plasmid at a concentration of 10 μg/ml and preincubated for 15 min prior to the addition of Lipofectin to a final concentration of 33 μg/ml. (*B*) The transformation frequency per μg (extrapolated from the data in *A*) plotted against increasing pIRT/URA4 concentration. Data from titrations in the presence of carrier DNA with no Lipofectin (△), and in the presence of Lipofectin with (□) or without (●) carrier DNA are presented. The data plotted represent means from duplicate transformations, except in the case of transformation in the absence of Lipofectin, which resulted from single transformations.

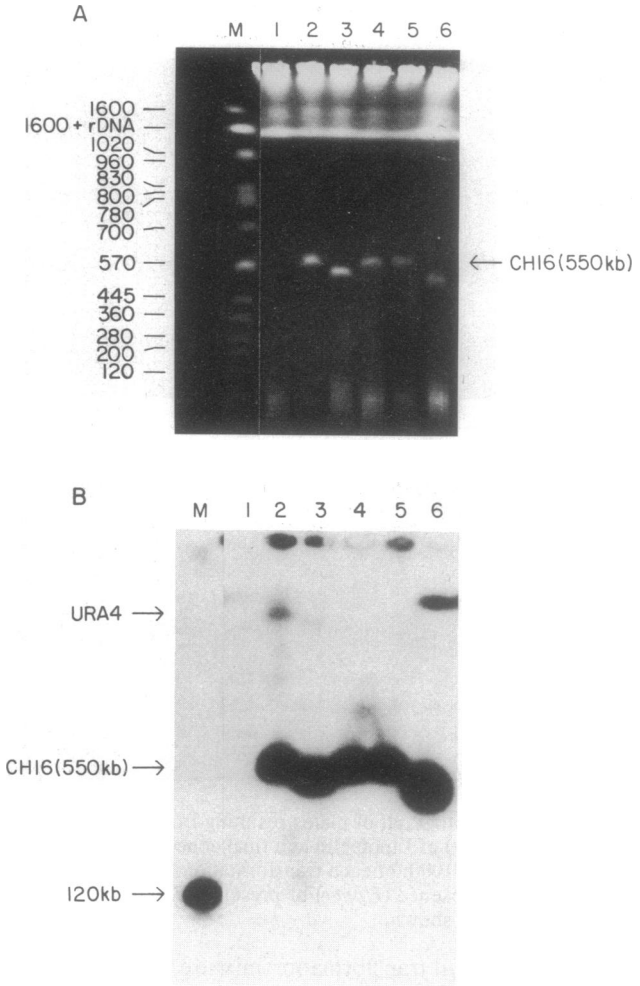

FIG. 4. Pulsed-field agarose gel of intact chromosomes from *Sc. pombe* Ch16 minichromosome donor and recipient strains and transformants. Chromosomal DNA prepared in agarose from *S. cerevisiae* strain AB1380-N7 containing a random human YAC of 120 kb (lane M), *Sc. pombe* strain SP813 (lane 1), *Sc. pombe* strain HM348-23R containing the minichromosome Ch16 (lane 2), and four SP813 Ade^+ Leu^+ Ch16 transformants (lanes 3–6) were separated by electrophoresis on a CHEF gel apparatus. (*A*) DNA in the CHEF gel after staining with ethidium bromide. (*B*) Autoradiograph of the DNA from the CHEF gel in *A* after transfer to Nytran and hybridization with ^{32}P-labeled pucURA4. The approximate sizes of the chromosomes in AB1380-N7 are shown in *A*. The signal corresponding to the *ura4* gene, the 120-kb human YAC in AB1380-N7, and the minichromosome Ch16 are indicated in *B*. The hybridization signal close to the top of lane 6 results from a translocation event between an additional copy of Ch16 and endogenous chromosome III (data not shown).

ture. All of these transformants were picked and examined for colony color. Four colonies were white, indicative of adenine prototrophy. This was confirmed by their ability to grow on minimal plates lacking adenine. Eighty-seven of the Leu^+ transformants were pink, indicating the presence of the donated minichromosome *ade6-216* allele. However, these grew poorly in the absence of adenine. Further analyses of these revealed that the minichromosomes had undergone translocation events with the endogenous chromosome III (data not shown). However, four white Ade^+ Leu^+ transformants were obtained, from which chromosomal-sized DNA was prepared and separated on a CHEF gel. As shown in Fig. 4*A*, all four of these transformants contain an additional minichromosome, similar in size to Ch16. This gel was transferred to a nylon membrane and hybridized with labeled pucURA4. Fig. 4*B* shows that this probe hybridizes to the minichromosome, since it contains plasmid DNA integrated with the *Leu2* gene close to the *fur1* locus. In addition, a weak band, corresponding to the *ura4* gene on chromosome III in the region of limited mobility, can be seen in HM348-23R but not in Sp813 or any of the minichromosome transformants. The absence of hybridization of the *ura4* gene to the Ch16 transformants verifies that this 550-kb minichromosome has indeed been transferred into SP813 by Lipofectin-enhanced transformation. Two of the transformants shown in Fig. 4 contain smaller minichromosomes than the original Ch16 DNA. This decrease in size most likely resulted from deletion events, the position and mechanism of which remains uncharacterized.

DISCUSSION

The results described here demonstrate that *Sc. pombe* transformation can be optimized to fulfill certain require-

ments. An incubation step of DNA with protoplasts and Lipofectin at 33 μg/ml increases the efficiency of transformation 10- to 50-fold. The inclusion of carrier DNA at 10 μg/ml increases efficiency of transformation when <250 pmol of plasmid per ml is used. Finally, this transformation procedure allows DNA molecules of >500 kb to be transferred into *Sc. pombe*.

The ability to introduce large linear DNA molecules into *Sc. pombe* could have several uses. If a suitable cloning system can be developed, it should be possible to clone large fragments of DNA from other species in *Sc. pombe*. The average size of DNA that might be cloned in *Sc. pombe* could exceed that which can currently be cloned in *S. cerevisiae* YACs.

Before introducing the minichromosome Ch16 into *Sc. pombe*, it was not known whether *Sc. pombe* telomeric repeats (22) could seed telomeres when introduced as DNA. In *S. cerevisiae*, telomeric repeats from many organisms (23–27) are efficient substrates for telomere addition *in vivo*. However, attempts to maintain linear molecules in *Sc. pombe* with either *Tetrahymena* or *S. cerevisiae* telomeric repeats resulted in circularization of the vectors in ≈90% of transformants (22, 28). This suggests that either *Sc. pombe* does not possess an efficient telomere recognition/replication machinery (e.g., telomerase; refs. 29 and 30) or *Tetrahymena* and *S. cerevisiae* telomeres do not function efficiently as templates for telomere replication in *Sc. pombe*.

The newly formed ends of minichromosome Ch16 have been shown to terminate with ≈300 base pairs of the *Sc. pombe* telomeric repeat (31). The fact that the minichromosome Ch16 replicates as a linear molecule when introduced here as naked DNA into *Sc. pombe* indicates that 300 base pairs of telomeric repeat is sufficient to be recognized and maintained as chromosomal termini on entry into the nucleus. It is likely that *Tetrahymena* and *S. cerevisiae* (22, 28) telomeres did not function efficiently in *Sc. pombe* because of the distinct difference in the sequence of *Sc. pombe* telomeres and, hence, in their recognition/replication machinery. The fact that *Sc. pombe* telomeres function when introduced into *Sc. pombe* as DNA is obviously important with respect to designing YAC-like cloning vectors.

Recently, it has been demonstrated that YACs in *S. cerevisiae* containing cloned *Sc. pombe* centromeres can be reintroduced into *Sc. pombe* on a vector containing a marker selectable in *Sc. pombe* (28). These YACs were stable in *Sc. pombe*, demonstrating that the cloned centromere retains its function upon reintroduction. Further characterization of *Sc. pombe* centromeres may allow a cloning vector to be designed that is maintained in a stable fashion because of the incorporation of a centromere. However, so long as selection is maintained, a centromere may not be an absolute necessity. In addition, an acentric cloning vector might allow heterologous centromeres to be identified by functional complementation in *Sc. pombe*.

Advantages to using *Sc. pombe* as a host for cloning large DNA include the fact that *Sc. pombe* chromosomes can replicate when introduced into mouse tissue culture cells (10). The ability to shuttle large fragments of mammalian or other DNA to and from cell lines or whole animals and yeasts will ultimately provide a powerful means for identifying and recovering genes of interest. Such technology should also provide an invaluable tool for the identification, isolation, and dissection of mammalian or other centromeres; for the construction of artificial chromosomes functional in mammalian cells; and for the manipulation of complex genomes.

I thank Charles DiComo for excellent technical assistance; David Beach and members of his laboratory for useful discussion, advice, and various materials; and Eric Richards and Rich Roberts for comments on the manuscript. I am especially grateful to Mitsuhiro Yanagida and members of his laboratory for supplying the minichromosome strains. I also thank Phil Hieter and Rakesh Anand for various helpful tips concerning the manipulation of large DNA. Lastly, I thank Sadie Arana for preparation of this manuscript.

1. Blackburn, E. H. (1985) *Trends Genet.* **1,** 8–12.
2. Burke, D. T., Carle, G. F. & Olson, M. V. (1987) *Science* **236,** 806–812.
3. Coulson, A., Waterston, R., Kiff, J., Sulston, J. & Kohara, Y. (1988) *Nature (London)* **335,** 184–186.
4. Guzman, P. & Ecker, J. R. (1988) *Nucleic Acids Res.* **16,** 11091–11105.
5. Garza, D., Ajioka, J. W., Burke, D. T. & Hartl, D. L. (1989) *Science* **246,** 641–646.
6. Anand, R., Villasante, A. & Tyler-Smith, C. (1989) *Nucleic Acids Res.* **17,** 3425–3433.
7. McCormick, M. K., Shero, J. H., Cheung, M. C., Kan, Y. W., Hieter, P. A. & Antonarakis, S. E. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9991–9995.
8. Vollrath, D. & Davis, R. W. (1987) *Nucleic Acids Res.* **15,** 7865–7876.
9. Fan, J.-B., Chikashige, Y., Smith, C. L., Niwa, O., Yanagida, M. & Cantor, C. R. (1989) *Nucleic Acids Res.* **17,** 2801–2818.
10. Allshire, R. C., Cranston, G., Gosden, J., Maule, J., Hastie, N. & Fantes, P. (1987) *Cell* **50,** 391–403.
11. Burgers, P. M. J. & Percival, K. J. (1987) *Anal. Biochem.* **163,** 391–397.
12. Beach, D. H. & Nurse, P. (1981) *Nature (London)* **280,** 140–142.
13. Beach, D. H., Piper, M. & Nurse, P. (1982) *Mol. Gen. Genet.* **187,** 326–329.
14. Niwa, O., Matsumoto, T., Chikashige, Y. & Yanagida, M. (1989) *EMBO J.* **8,** 3045–3052.
15. Beach, D. H., Rodgers, L. & Gould, J. (1983) *Curr. Genet.* **10,** 297–311.
16. McCleod, M., Stein, M. & Beach, D. (1987) *EMBO J.* **6,** 729–736.
17. Niwa, O., Matsumoto, T. & Yanagida, M. (1986) *Mol. Gen. Genet.* **203,** 397–405.
18. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
19. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
20. Felgener, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. & Danielsen, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7413–7417.
21. Felgener, P. L. & Holm, M. (1989) *Focus* **11,** 21–25.
22. Sugawara, N. & Szostak, J. (1986) *Yeast* **2,** Suppl., 373 (abstr.).
23. Cross, S. H., Allshire, R. C., McKay, S. J., McGill, N. I. & Cooke, H. J. (1989) *Nature (London)* **338,** 771–774.
24. Brown, W. R. A. (1989) *Nature (London)* **338,** 774–776.
25. Szostak, J. W. & Blackburn, E. (1982) *Cell* **29,** 245–255.
26. Dani, G. M. & Zakian, V. A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3406–3410.
27. Pluta, A. F., Dani, G. M., Spear, B. B. & Zakian, V. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1475–1479.
28. Hahnenberger, K. M., Baum, M. P., Polizzi, C. M., Carbon, J. & Clarke, L. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 577–581.
29. Greider, C. & Blackburn, E. H. (1989) *Nature (London)* **337,** 331–337.
30. Morin, G. B. (1989) *Cell* **59,** 521–529.
31. Matsumoto, T., Fukui, K., Niwa, O., Sugawara, N., Szostak, J. W. & Yanagida, M. (1987) *Mol. Cell. Biol.* **7,** 4424–4430.