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Behavior of chromosomes after meiosis in *Coprinus cinereus*

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The movement was investigated of a specific chromosome in the F_1 progeny of the basidiomycete *Coprinus cinereus*. We focussed our attention on the smallest chromosome of the 5302 strain. We first constructed a chromosome-specific library and screened it with a chromosome-specific clone, pRC 1. The pRC 1 probe hybridized only with the smallest chromosome of the 5302 strain, and it detected one band of different mobility in two parental strains. In the F_1 progeny, the probe hybridized with one to three chromosomes. Most of the hybridized chromosomes in the F_1 progeny were positioned in terms of mobility between the hybridized chromosomes of the two parental strains. Therefore, they were probably generated by meiotic recombination between homologous chromosomes of different sizes.

Key Words——chromosome-specific clone; F_1 progeny; meiotic recombination.

The study of fungal genetics has been revolutionized by the development of pulsed-field gel electrophoresis (PFGE) and its application to questions related to the size, organization and stability of fungal genomes (for reviews, see Mills and McClus key, 1990; Skinner et al., 1991). In many fungi, the electro phoretic analysis of chromosomes has revealed extensive chromosome length polymorphism (CLP; Mills and McCluskey, 1990; Skinner et al., 1991; Pukkila and Casselton, 1991; Zolan et al., 1992; Arima and Morinaga, 1995). Such CLP may reflect translocations, of which several clear cases have been reported (Tzeng et al., 1992). However, the causes of CLP have not been clarified in many cases.

The basidiomycete *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray has proven to be a useful model system for analysis of the meiotic process (for review, see Pukkila and Casselton,1991). The nuclear genome of *C. cinereus* is approximately 37,500 kb in length (Dutta, 1974), and *C. cinereus* has 13 chromosomes (Pukkila and Lu, 1985), which range in size from 1 to 5 megabases (Mb). The CLP among strains of *C. cinereus* has been demonstrated by PFGE (Pukkila and Casselton, 1991; Zolan et al., 1992,1994; Arima and Morinaga, 1995). The electrophoretic karyotype of each strain of *C. cinereus* is mitotically stable. However, the chromosome encoding the repeat array of genes for ribosomal RNA varies in size in mitotic division (Pukkila and Skrzynia, 1993).

Although the electrophoretic karyotype is stable in mitotic division, F_1 progeny can be isolated by crossing strains with different karyotypes to allow the isolation of unique karyotypes (Arima and Morinaga, 1995). This study was designed to observe the movements of a specific chromosome. This investigation is a first step towards a better understanding of the mechanism of generation of CLP.

Materials and Methods

Strains of *C. cinereus* and culture conditions The *Coprinus cinereus* monokaryon 5302 (Kamada et al., 1993) was used to construct the chromosome-specific library. The PR series of F_1 progeny was obtained by crossing strains of *C. cinereus* 5302 and Dd 13 (from Dr.T. Kamada, Okayama University, Japan). The strains used in this study are described in Table 1. Potato-sucrose medium (Morinaga et al., 1985) was used for vegetative growth and formation of fruiting bodies.

Strain of *Escherichia coli* and medium *Escherichia coli* strain JM 109 (Yanisch-Perron et al., 1985) was used as the host for cloning of plasmids. *E. coli* cells were routinely grown in Luria-Bertani medium, as described by Maniatis et al. (1982).

Manipulation of DNA Standard protocols were used for digestion with restriction enzymes, ligation, agarose gel electrophoresis and Southern blotting (Maniatis et al., 1982). Gels were blotted onto Hybond-N membranes (Amersham). Hybridization was performed with a non-radioactive digoxygenin-dUTP-labeled DNA probe. Hybridization was carried out according to the recommendations of the manufacturer of the label (Boehringer Mannheim). Plasmid DNA was isolated by alkaline lysis, as described by Birnboim and Doly (1979). Cloning of plasmids and bacterial transformation were performed by

Table 1. Strains.

Strain	Mating type	Genotype	Used as
5302	*A2B2*	Wild type	Parent
Dd13	*A12B12*	Wild type	Parent
PR series		Wild type	F_1 progeny

standard procedures (Maniatis et al., 1982). Genomic inserts for use as probes were purified by two cycles of agarose gel electrophoresis.

Chromosome plugs and pulsed-field gel electrophoresis Plugs of intact chromosomes of *C. cinereus* were prepared as described elsewhere (Arima and Morinaga, 1995) and analyzed by contour-clamped homogeneous electric field (CHEF) gel electrophoresis in a system from Bio Craft (model BS-80; Bio Craft,Tokyo). Conditions for CHEF gel electrophoresis were those described previously (Arima and Morinaga, 1995).

Construction of the chromosome-specific library The chromosome-specific library was constructed in the pUC18 vector (Gibco-BRL). Digestion with *Eco* RI and dephosphorylation of the vector were performed according to the recommendations of the manufacturer (Boehringer Mannheim). Inserts were prepared by digestion of the chromosome of interest with *Eco* RI. Fragments were electroeluted from gels after electrophoresis and ligated to pUC18 that had been cleaved with *Eco* RI. The methods used for cloning were essentially those described by Zolan et al. (1992). One-half of the ligation mixture was used to transform *Esherichia coli* JM 109. Recombinants were selected on LB agar that contained 100 μg ml^{-1} ampicillin (Sigma).

Results

Electrophoretic karyotype Figure 1, which is from Arima and Morinaga (1995), shows that the two parental strains (5302 and Dd 13) and the dikaryon derived from the cross between these parental strains exhibit different chromosomal patterns after CHEF gel electrophoresis. We investigated the inheritance of this chromosome length polymorphism. We crossed the 5302 strain with the Dd 13 strain and isolated F_1 progeny from one of the fruiting bodies. The electrophoretic karyotype of each F_1 progeny was unique (Figs. 2a, 2b), and several novel

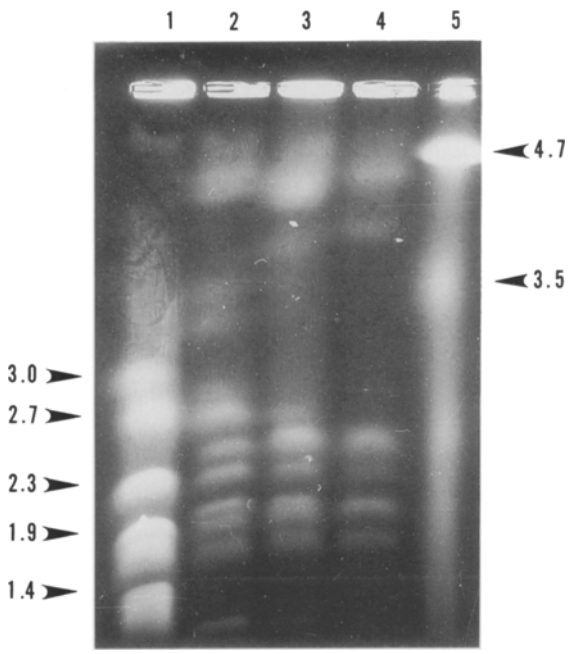

Fig. 1. Electrophoretic karyotypes of the parental strains of *Coprinus cinereus*.
Lane 1, *Candida albicans* (ATCC14053); lane 2, strain 5302; lane 3, 5302×Dd 13; Lane 4, strain Dd 13; lane 5, *Schizosaccharomyces pombe* (972). Molecular size markers in the left and right lanes were derived from *C. albicans* and *S. pombe* and their sizes are indicated in megabase pairs.

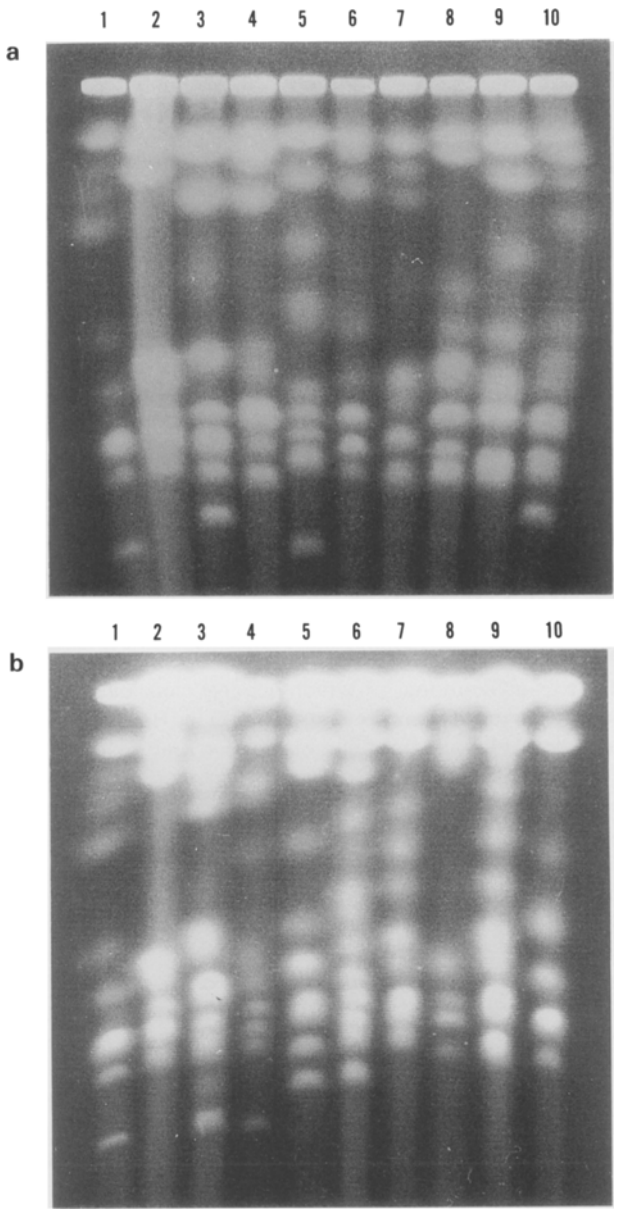

Fig. 2. Electrophoretic karyotypes for parental strains and F_1 progeny (PR series) from a cross between *C. cinereus* 5302 and Dd 13.
a. Lane 1, 5302; lane 2, Dd 13; lane 3, PR-1; lane 4, PR-2; lane 5, PR-3; lane 6, PR-4; lane 7, PR-5; lane 8, PR-6, lane 9, PR-7; lane 10, PR-8. b. Lane 1, 5302; lane 2, Dd 13; lane 3, PR-9; lane 4, PR-10; lane 5, PR-11; lane 6, PR-12; lane 7, PR-13; lane 8, PR-14; lane 9, PR-15; lane 10, PR-16.

electrophoretic karyotypes were found. In later experiments, we focussed on the smallest chromosome (about 1 Mb) in the 5302 strain, since this chromosome was associated with the most conspicuous chromosome length polymorphism in the two parental strains (Fig. 1). This chromosome was observed in the PR-3, PR-9 and PR-10 strains of the F_1 progeny (Figs. 2a, 2b).

Screening of the chromosome-specific library We constructed a chromosome-specific library from the smallest chromosome of the 5302 strain. We selected 20 random clones from the library, and these were analyzed by hybridization to ascertain whether they were specific or nonspecific for the smallest chromosome of the 5302 strain. Most of the clones, for example, pRC-1 and pRC-2, yielded only a signal that was specific for the smallest chromo some of the 5302 strain (Fig. 3). In spite of extraction of only the smallest chromosome of the 5302 strain, two clones (pRC-3 and pRC-4) hybridized with another chromosome (Fig. 3). The clone pRC-5 detected multiple bands including the smallest chromosome (Fig. 3). Thus, these clones could be divided into three classes. We used a clone that hybridized only with the smallest chromosome of the 5302 strain in the next experiment.

Movement of the smallest chromosome We monitored the movement of the smallest chromosome using pRC-1, which hybridized only with the smallest chromosome of the 5302 strain (Figs. 4a, 4b). The probe hybridized with one chromosome in both parental strains. In the F_1 progeny, however, the probe hybridized with one to three chromosomes and hybridization patterns were variable (Figs. 4a, 4b). Most of these strains yielded novel hybridization patterns. They were classified according to these results. Two strains (PR-3 and PR-10) gave the same result as the 5302 strain. Three strains (PR-13, PR-14 and PR-16) gave a result identical to that obtained with the Dd 13 strain. In eight strains (PR-1, PR-4, PR-5, PR-6, PR-7, PR-8, PR-11 and PR-12), the probe hybridized with one chromosome. This chromosome was located between those of the hybridizing chromosomes of the two parental strains. In the PR-2 strain, the probe hybridized with one chromosome that was longer than the hybridized chromosome in the Dd 13 strain. We detected two bands, which were equivalent to the parental type (5302 strain) and a new type, in the PR-9 strain. In the PR-15 strain, the probe hybridized with three chromosomes: the parental type (Dd 13 strain) and two new types. One of these chromosomes was bigger than

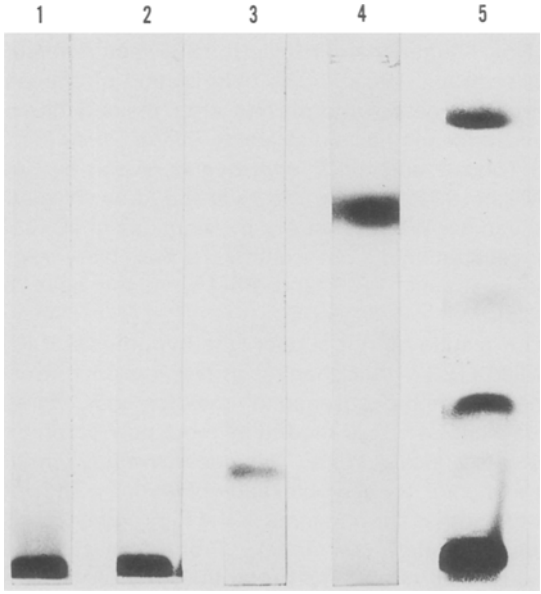

Fig. 3. Screening for chromosome-specific clones. CHEF-resolved chromosomal DNA of *C. cinereus* 5302 strain after Southern blotting and hybridizations with (lanes 1 to 5, respectively) probes pRC-1, pRC-2, pRC-3, pRC-4, and pRC-5.

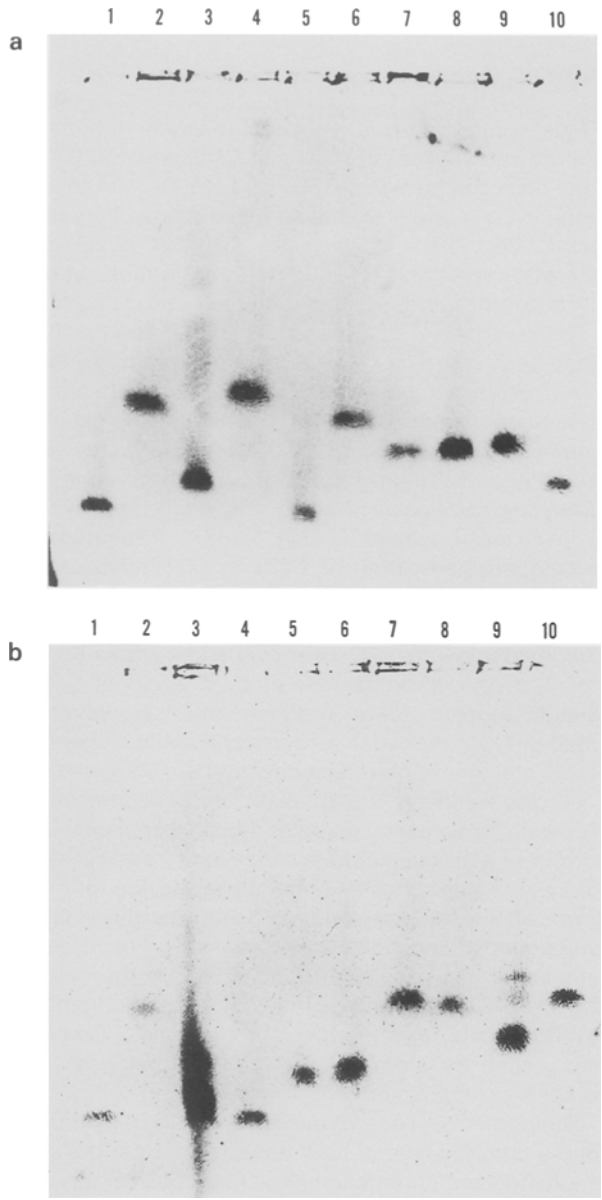

Fig. 4. Hybridization of CHEF-separated chromosomes of parental and F_1 progeny strains from Figs. 2a and 2b probed with pRC-1. See legend to Fig. 2 for details of lanes.

the hybridized chromosome in the Dd 13 strain.

Discussion

CHEF gel electrophoresis was used to examine the electrophoretic karyotypes of strains of *C. cinereus* 5302, Dd 13, the dikaryon between 5302 and Dd 13, and their F_1 progeny. Each strain examined had a unique karyotype (Figs. 1, 2a, 2b). In particular, several novel electrophoretic karyotypes which differed from those of both parental strains were found among the F_1 progeny.

The mechanism of generation of CLP has not been clarified in *C. cinereus*. The human pathogen *Plasmodium falciparum* exhibits extensive CLPs, which map to subtelomeric repeats that are present on some chromosomes (Walliker et al., 1987). Moreover, highly variable subtelomeric repeats have also been observed in *Saccharomyces cerevisiae* Meyen ex E.C. Hansen and *Drosophila melanogaster* (Zakian, 1989). It seemed possible that the generation of CLPs in *C. cinereus* might have been due to changes in tandem repeats, or telomeric, subtelomeric or interstitial sites. We have examined the telomeric repeats in *C. cinereus*, and it appeared that the ends of the chromosomes of *C. cinereus* also have telomeric repeats (data not shown). However, it seemed unlikely that such arrays alone could explain the dynamic changes in chromosomes that we observed.

We focussed on the smallest chromosome of the 5302 strain for an examination of dynamic changes. We constructed a chromosome-specific library and screened progeny with a chromosome-specific probe (Fig. 3). The majority of the clones hybridized with only the smallest chromosome of the 5302 strain, perhaps because this chromosome has a peculiar sequence. In the case of pRC 3 and pRC 4, the probes may have been contaminated by fragments from another chromosome. Common regions, such as promoter and operator regions, may have been included in pRC-5.

Although the probe derived from pRC-1 hybridized with one chromosome in both parental strains, the sizes of the hybridized chromosomes varied considerably. In the F_1 progeny, hybridization with one to three chromosomes was observed (Figs. 4a, 4b). These strains showed novel hybridization patterns, but their hybridized bands were located between those of the two parental strains. Therefore, they were most likely formed by meiotic recombination between homologous chromosomes of different sizes. A cross between strains with different karyotypes may produce progeny with a chromosome of a new size. From these considerations, we constructed a simple model of meiotic recombination, as shown in Fig. 5.

When we used pRC-3 as probe, we detected one band at the same position as both parental strains. In the F_1 progeny, the probe hybridized with one chromosome that was located at roughly the same position in the analysis of both parental strains (data not shown). From these results, it seems that both parental strains with homologous chromosomes of the same size may have distributed a chromosome of about the same size to

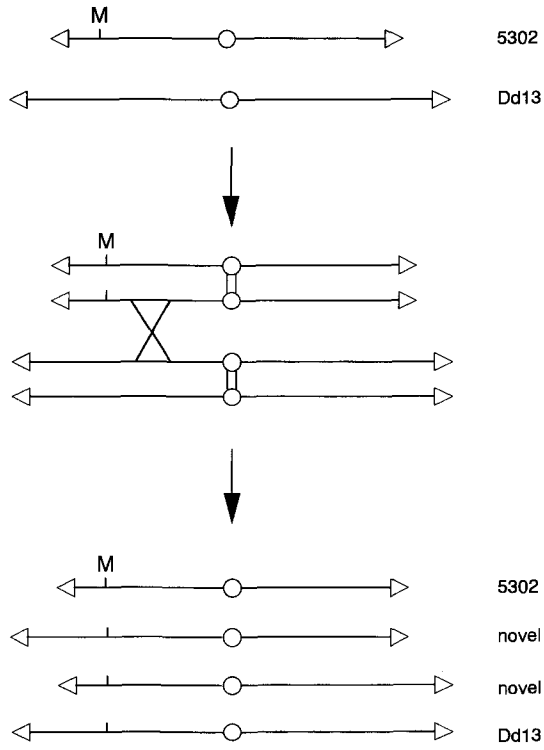

Fig. 5. Simple model of the proposed meiotic recombination. M represents the chromosome-specific marker pRC 1.

the F_1 progeny by meiotic recombination. This result is consistent with our model of meiotic recombination.

A similar phenomenon of formation of a novel CLP pattern and new size of chromosome after meiosis has been identified in *S. cerevisiae* (Ono and Ishino-Arao, 1988), *Ustilago maydis* (de Candolle) Corda (Kinscherf and Leong, 1988), *Leptosphaeria maculans* (Desm.) Ces. & de Not. (Plummer and Howlett, 1993) and *P. faliparum* (Walliker et al., 1987). The contribution of aneuploidy to chromosome polymorphisms after meiosis has been discussed for the haploid strain of *Tilletia caries* (de Candolle) Tulasne and for *T. controversa* Kuehn by Russell and Mills (1993) and for *Yarrowia lipolytica* (Wickerham et al.) van der Walt & von Arx by Naumova et al. (1993). Our investigation did not exclude the possibility that two progeny strains, PR-9 and PR-15, might have been aneuploid.

Our results do not support the hypothesis of Kistler and Miao (1992) that changes in chromosome structure are neutral in the absence of meiosis and, therefore, asexual species would tend to be more polymorphic than those with a sexual stage. However, we have provided clear evidence for meiotic recombination. The movement of another chromosome in the F_1 progeny should allow us to validate our model. From such studies, we should be able to obtain a better insight into the generation of CLP in *C. cinereus*.

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