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Dispersed Repetitive DNA Sequence of *Mucor racemosus*

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A dispersed repetitive DNA sequence has been identified within the genome of the fungus *Mucor racemosus*. Recombinant phage clones, as well as a plasmid harboring the sequence, have been isolated. Examination of cloned fragments comprising part of the repetitive sequence has led to a partial characterization of the element. The sequence has been detected in other *Mucor* species, and although the apparent number and chromosomal position of the repetitive sequence vary from strain to strain, it is clear that at least portions of the element have been conserved.

Dispersed repetitive DNA sequences are ubiquitous in eucaryotic genomic DNA. Some of these elements are transposable (3, 4, 15) and in addition may be important in influencing gene expression (4, 9, 13) as well as in promoting genetic diversity by mediating genetic rearrangements (1, 10, 16). For example, Ty1, a 5.9-kilobase (kb) transposable element of *Saccharomyces cerevisiae*, affects the expression of certain genes located immediately downstream from the point of Ty1 insertion (9, 14). It is also clear that transcription of Ty1 itself is, at least in part, under mating-type control (8). Likewise, the number of transcripts homologous to the *Dictyostelium discoideum* transposon, DIRS-1, increase approximately 20-fold during development compared with the amount of DIRS-1-specific RNA present in vegetative cells (18).

We recently identified and isolated a dispersed repetitive DNA sequence from the genomic DNA of *Mucor racemosus*. The sequence was detected while we were examining recombinant plasmids initially isolated from a pBR322-*M. racemosus* gene library on the basis of their harboring *M. racemosus* tRNA genes. When a number of the plasmids were digested concomitantly with restriction endonucleases *Hin*dIII and *Bam*HI and then analyzed by agarose gel electrophoresis, it was observed that several of the *Hin*dIII-*Bam*HI-generated fragments, ranging in size from 0.8 to 0.65 kb, were common to some of the plasmids. Blotting experiments revealed that tRNA genes were not contained on the common fragments, and the possibility that the fragments might comprise a portion of an *M. racemosus* dispersed repetitive DNA sequence was then investigated. The appropriate fragments were electroeluted from the gel and then labeled with [α-^{32}P]dGTP by nick translation (12). Each fragment was then used in cross-hybridization experiments against *Hin*dIII-*Bam*HI-digested plasmid DNA blotted to nitrocellulose (17) and in hybridization experiments against *Hin*dIII-digested genomic DNA. Only self-homology was observed in cross-hybridization experiments, indicating that common sequences were not shared by the plasmids; however, hybridization of a 0.8-kb *Hin*dIII-*Bam*HI-generated fragment of plasmid pMu140 (Fig. 1) demonstrated that it was represented a number of times in *M. racemosus* genomic DNA (Fig. 2). The repeated sequence also was not found to be generally associated with *M. racemosus* tRNA genes. In particular, an additional 75 *M. racemosus* tRNA-containing clones were examined (average insert = 8 kb),

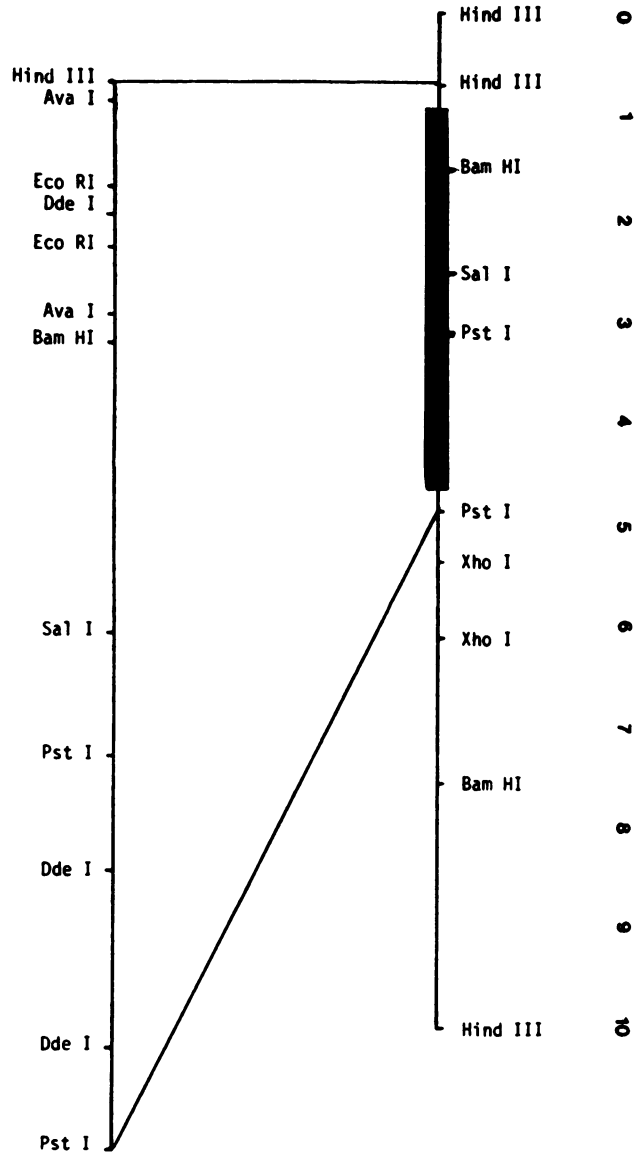

FIG. 1. Restriction map of the *M. racemosus* DNA insert in plasmid pMu140. The insert shows the region containing MuR in greater detail. The tRNA gene(s) contained on the insert is located on the terminal *Bam*HI-*Hin*dIII fragment. Map distances are shown in kilobase pairs.

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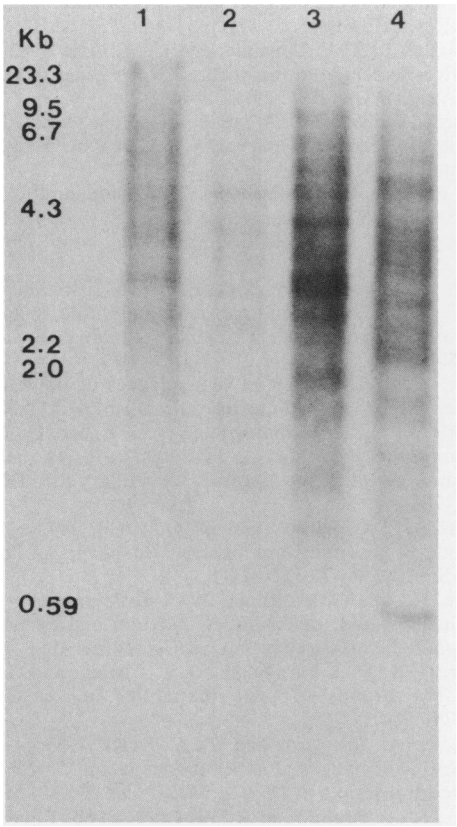

FIG. 2. Distribution of the *M. racemosus* repeated DNA sequence in other *Mucor* species. DNA was isolated from the various strains as described previously (5). Approximately 3 μg of DNA from each sample was digested with *Hin*dIII and subjected to electrophoresis in a 1.2% agarose gel. After transfer to nitrocellulose, the DNAs were hybridized to the ^{32}P-labeled 0.8-kb fragment. The hybridization spectrum was visualized by autoradiography. Lanes: 1, *M. genevensis* DNA; 2, *M. hiemalis* DNA; 3, *M. mucedo* DNA; 4, *M. racemosus* DNA. Size markers are indicated in kilobase pairs.

and none carried sequences homologous to the fragment, as judged by colony hybridization experiments. Therefore, the data suggest that the repetitive sequence is not analogous to sigma, the *S. cerevisiae* repetitive DNA sequence (6).

Experiments were performed to define the approximate size and repetition frequency of the repetitive DNA sequence, designated MuR. The boundaries of MuR were localized by isolating fragments adjacent to the 0.8-kb *Hin*dIII-*Bam*HI fragment (between 0.8 and 1.6 kb on the restriction map of pMu140 [Fig. 1]), labeling the fragments as described above, and finally utilizing the labeled fragments as probes against *Hin*dIII-digested *M. racemosus* DNA. The termini of MuR lay near the *Hin*dIII site at 0.8 kb and the *Pst*I site at 3.3 kb on the pMu140 restriction map. Furthermore, results from dot-blot hybridization experiments indicate that MuR is repeated about 25 to 35 times in *M. racemosus* genomic DNA (data not shown).

Additional copies of MuR were isolated to gain further insight on the reiteration of the sequence. An *M. racemosus* λ Charon 4A gene library, constructed with genomic DNA partially digested with *Eco*RI, was screened by plaque hybridization for recombinant phage harboring sequences homologous to MuR (2). Again, the 0.8-kb *Hin*dIII-*Bam*HI fragment of pMu140 was used as the probe. Positive plaques were chosen, and the putative recombinant phages were subjected to three successive cycles of plaque purification. DNA was extracted from six of the phage isolates and analyzed by blotting experiments (Fig. 3). The individual isolates were all distinct from each other and contained either one or two *Eco*RI-generated fragments homologous to the probe. Further restriction analysis of these fragments indicated that sequence heterogeneity exists between individual copies of MuR. The evidence also suggests that copies of MuR are not highly clustered.

Other investigations have been performed to determine whether elements similar to MuR are present in the genomic DNA of related *Mucor* species. In this regard, DNA from *M. genevensis*, *M. hiemalis*, and *M. mucedo* was obtained and probed for sequences homologous to MuR (Fig. 2). Sequences related to MuR were present in the genomic DNA of each isolate examined. In addition, similar observations

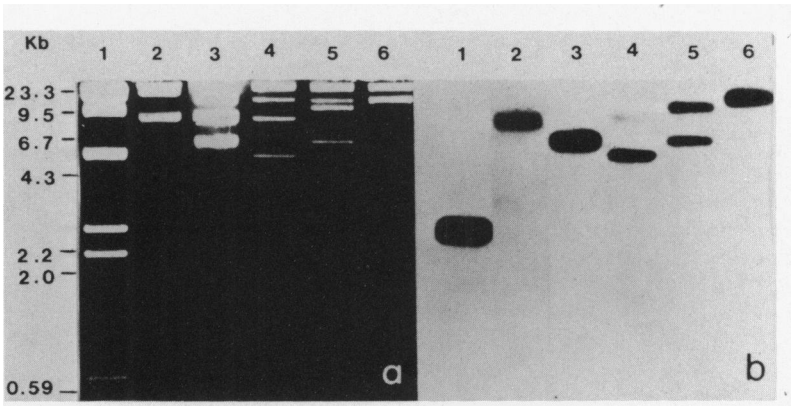

FIG. 3. *Eco*RI restriction patterns (a) and hybridization profiles (b) of DNA from six phage clones isolated on the basis of containing sequences homologous to the 0.8-kb *Hin*dIII-*Bam*HI fragment of pMu140. The two largest *Eco*RI-generated fragments in panel a represent vector arms; the other fragments represent *M. racemosus* DNA. The phage library was constructed as described by Maniatis et al. (11), with *M. racemosus* DNA partially digested with *Eco*RI. Average insert size was approximately 15 kb. The plaque hybridization technique of Benton and Davis (2) was employed to isolate appropriate recombinant phage. Plaque hybridizations were performed in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 10× Denhardt solution (7) and 0.1% sodium dodecyl sulfate for 16 to 24 h at 65°C in heat-sealable bags. The desired phage plaques were identified after autoradiography, plaque purified, and finally propagated as described previously (11). Transfer of restriction fragments to nitrocellulose was done by the method of Southern (17). The probe and the hybridization conditions were identical to those for plaque hybridization. After hybridization, filters were washed in 5× SSC for 60 min at 65°C and then three times at room temperature in 2× SSC.

were made when *Mucor* spp. DNAs were digested with either *Eco*RI or *Pst*I. Thus, although the apparent number and chromosomal distribution of the MuR family of repetitive sequences vary among strains, portions of the element have been conserved.

The functional significance of MuR remains obscure. It is not yet known whether MuR is transposable, but analysis of the element at the DNA sequence level should reveal whether MuR shares the structural characteristics of other transposable elements (3, 13). The fact that MuR sequences are present in several other *Mucor* species suggests that the element might be exploited in the development of an integrative cloning vehicle for use with this group of filamentous fungi. Investigations are continuing to test this possibility.

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