Mapping the Heterogeneous DNA Region That Determines the Nine Aα Mating-Type Specificities of *Schizophyllum commune*

Charles A. Specht,*[,1] Mary M. Stankis,[†,2] Charles P. Novotny[†] and Robert C. Ullrich*

**Department of Botany and [†] Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, Vermont 05405*

Manuscript received December 18, 1991
Accepted for publication March 12, 1994

ABSTRACT

Classical genetic studies identified nine mating-type specificities at the *Aα* locus of the Basidiomycete fungus *Schizophyllum commune*. We have used Southern blot hybridizations to generate *Eco*RI restriction maps of the *Aα* locus for 18 strains, including all nine specificities. Aα1, Aα3 and Aα4 DNA was subcloned from three cosmids and used as probes. A unique region of DNA was found for each of the three cloned specificities. Hybridization was detected in this region only if the probe(s) and the blotted genomic DNAs were from strains with the same Aα specificity. DNAs from strains with the same Aα specificity hybridize regardless of geographic origin, but DNAs from strains with different Aα specificities do not cross-hybridize. The results demonstrate two size classes of unique Aα DNA. This unique DNA is about 4.5 kb in Aα1 strains and about 7.0–8.5 kb in other strains. Transcription regulators Z and Y, which were deduced previously from the DNA sequence of the *Aα1*, *Aα3* and *Aα4* loci, are probably encoded by all non-*Aα1* loci. The smaller *Aα1* loci appear to encode only Y and lack sequence for Z. No evidence was found for a locus that encodes only Z. The lack of hybridization detected between *Aα* loci with different specificities suggests that the evolution of *Aα* has resulted from extensive sequence divergence.

SCHIZOPHYLLUM commune is a model organism for the study of tetrapolar mating type in Basidiomycetes (reviewed by RAPER 1983, 1988). Mating type in *S. commune* was initially thought to be determined by two unlinked loci termed the *A* and *B* factors (KNIEP 1920). Subsequently, each factor was found to consist of two linked loci designated *Aα* and *Aβ*, and *Bα* and *Bβ*, respectively (PAPAZIAN 1951; KOLTIN *et al.*. 1967). For two homokaryons to be sexually compatible, *i.e.*, differentiate into fertile dikaryotic mycelium after fusion, the two homokaryons must have different *A* and *B* factors. Differences at either α or β, or both are sufficient for differentiation (PAPAZIAN 1951; KOLTIN *et al.* 1967). Genetic studies show 9 Aα, an estimated 32 Aβ, 9 Bα, and 9 Bβ specificities exist in nature. The combinations of α and β specificities constitute potentially 23,328 different mating types of *S. commune*.

The structure of the *Aα* locus has been determined for three specificities (STANKIS *et al.* 1992). The Aα1, Aα3 and Aα4 specificities are dissimilar in DNA sequence, but the *Aα* alleles are clearly homologous. Thus, they cannot be termed idiomorphs (METZENBERG 1990). *Aα3* and *Aα4* loci each encodes an allele of two putative transcription regulators termed Z and Y. *Aα1* encodes an allele of Y, but lacks sequence for Z. DNA sequences that flank the three loci show about 90% identity, whereas comparable Aα sequences show only small regions with 70–80% identity; the majority is about 50% identical (STANKIS *et al.* 1992).

Although the structure of the *Aα* locus in three strains with Aα1, Aα3 and Aα4 specificities had been determined (STANKIS *et al.*. 1992), nothing was known about the structures of *Aα* encoding the remaining six specificities (Aα2, Aα5–Aα9). The degree of natural variation that might exist in the structure of *Aα* in strains with the same specificity, but from different geographic regions was also unknown. To gain a more complete understanding of the *Aα* locus we have used probes from the three cloned *Aα* loci and from flanking regions in Southern blot hybridizations to construct restriction maps of the *Aα* locus of 18 strains including all nine Aα specificities. Several of the loci were from strains with the same Aα specificity in order to assess the degree of natural variation in *Aα*. Seven *Aα1* loci were mapped in order to determine if each locus encodes only the Y protein.

MATERIALS AND METHODS

***S. commune* strains and their culture:** Strains used in this study are from the collection of J. R. RAPER, Harvard University, currently maintained at the University of Vermont (Table 1). The Aα specificity of each strain was previously determined (RAPER *et al.* 1960). Genotypes of strains used for DNA-mediated transformations and matings with transformants are described (Table 2). Mycelia were grown using complete yeast extract medium (CYM, HOFFMAN and RAPER 1974) or CYM supplemented with 4 mM tryptophan (CYMT).

Cloning of the *Aα3* locus: The *Aα3* locus was isolated essentially as described for the *Aα1* locus (GIASSON *et al.* 1989).

[1] Present address: Center for Cancer Research, Massachusetts, Institute of Technology, Cambridge, Massachusetts 02139.
[2] Present address: Myco Pharmaceuticals Inc., 1 Kendall Square, Cambridge, Massachusetts 02139.

TABLE 1

Strains of *S. commune* used in Southern blot hybridizations

Strain	*Aα*	*Aβ*	Designation for this study	Origin
UVM 4-39	1	1	1a[a]	Massachusetts
UVM 1-15	1	9	1b	?[b]
UVM 1-23	1	5	1c	Massachusetts
UVM 1-44	1	3	1d	?
UVM 1-59	1	5	1e	Pennsylvania
UVM 1-103	1	15	1f	Costa Rica
UVM 1-113	1	7	1g	North Carolina
UVM 2-19	2	1	2	Australia
UVM 1-71	3	1	3a[a]	Brazil
UVM 1-41	3	5	3b	Massachusetts
UVM 4-40	4	6	4a[a]	Massachusetts
UVM 1-2	4	7	4b	Illinois
UVM 1-87	4	16	4c	North Borneo
UVM 1-43	5	1	5a	Alabama
V 4-5	5	7	5b	?
UVM 2-21	6	1	6	New Guinea
UVM 2-22	7	1	7a	Massachusetts
UVM 1-116	7	3	7b	?
UVM 2-23	8	1	8	Mozambique
UVM 2-24	9	1	9	Russia

[a] The *Aα* locus from each of these strains has been cloned and are being sequenced.
[b] ? indicates that origin is unrecorded.

TABLE 2

***S. commune* strains used as recipients in transformation or strains for the analysis of transformants in the isolation of the *Aα3* locus**

Strain	Genotype[a]
UVM 8-106	*Aα3 Aβ5 Bαx Bβx trp1 ura1*
UVM 12-43	*Aα3 Aβ5 Bα2 Bβ2 ura1*
UVM 8-87	*Aα5 Aβ7 Bαx Bβx trp1*
UVM 8-108	*Aα5 Aβ7 Bα3 Bβ2 trp1*

[a] "x" has been used when the specificity of the individual *Bα* or *Bβ* locus is unknown. The *B* factor determined by *Bαx, Bβx* is different from the *B* factor determined by *Bα2, Bβ2.*

A genomic library was constructed with DNA isolated from an Aα3 strain, UVM 1-71. DNA (partially digested with *Sau*3A) was ligated to *Bam*HI cut cosmid vector pTC20, which contains the Schizophyllum *TRP1* gene as a selectable marker for transformations (GIASSON *et al.* 1989). DNA was packaged using lambda extracts (Stratagene). *Escherichia coli* strain *DH1* was transfected, and ampicillin resistant colonies were selected.

DNA flanking the *Aα* locus exhibits high sequence identity among strains. This DNA from an Aα4 cosmid was used to identify clones bearing DNA from the Aα3 strain by colony hybridization. Cosmids were shown to have Aα3 mating-type specificity by DNA-mediated transformation of homokaryotic strains. Recipients had either Aα3 or non-Aα3 mating-type specificities. Trp^+ transformants were paired with tester strains that had the same *A* factor and different *B* factors as the recipient. The pairings were scored microscopically for formation of dikaryotic hyphae.

Subcloning: Restriction fragments were subcloned from cosmids into *pUC18* or *pIBI30* and transformed into *E. coli* strains *DH5α, JA228, HB101* or *NM522* by standard methods (SAMBROOK *et al.* 1989).

DNA extractions: Purification of total genomic DNA from Schizophyllum mycelium has been described (SPECHT *et al.* 1982). Plasmid DNA was extracted from *E. coli* by the cleared lysate method (CLEWELL 1972) and purified by two rounds of centrifugation in CsCl-ethidium bromide gradients. Cloned DNA fragments to be used as probes in Southern hybridizations were purified from agarose gels following electrophoresis (SAMBROOK *et al.* 1989).

Southern blot hybridizations: Genomic DNA (1–2 μg) was restricted with *Eco*RI; electrophoresed in 1% agarose and transferred to Zeta-probe membranes (Bio-Rad) as described by SOUTHERN 1975. *Hin*dIII digested λDNA served as the standard for estimating the size of DNA fragments. Cloned DNA fragments to be used as probes were labeled with $[\alpha P^{32}]$dCTP by random primer synthesis (Pharmacia) to specific activities *ca.* 1×10^8 cpm/μg. Hybridizations were for 20 h at 65° in 5 × SSC, pH 7.0; 5 × Denhardt's solution; 0.1% sodium dodecyl sulfate (SDS); 0.5% Carnation powdered milk; 100 μg/ml denatured, herring sperm DNA; and *ca.* 2×10^6 cpm/ml of labeled probe. Exposures to X-ray film were made after filters were rinsed three times for 20 min in 2 × SSC, 0.1% sodium pyrophosphate, 0.1% SDS at 65° (low stringency rinse) and again following two rinses for 20 min in 0.1 × SSC, 0.1% sodium pyrophosphate, 0.1% SDS at 65° (high stringency rinse).

DNA sequence comparisons: DNA sequence alignments were made to coincide with amino acid alignments of respective coding regions. Alignments were facilitated by the Bestfit program (GCG, Wisconsin). A percent identity was calculated every 50 bases along each alignment.

RESULTS

Cloning the *Aα3* locus: Three cosmid clones with overlapping sequences were isolated from the Aα3 genomic library by probing with DNA that flanks the *Aα4* locus. DNA-mediated transformations with these cosmids were done using homokaryotic recipients. Strain UVM 8-106 with Aα3 specificity yielded 120 Trp^+ transformants with no alteration of *A* factor activity as evidenced by pairing with UVM 12-43 (same *A* factor, different *B* factor as UVM 8–106). Homokaryotic strain UVM 8-108 with Aα5 specificity yielded 30 Trp^+ transformants of which 10 mated with strain UVM 8-87 carrying *Aα* and *Aβ* identical to the transformation recipient. We conclude from these results that the *Aα3* locus is contained within each of the cosmids that were isolated.

Restriction maps of *Aα* loci as determined by Southern blot hybridizations: *Eco*RI restriction maps of the three *Aα* loci that were cloned (*Aα1* and *Aα4*, GIASSON *et al.* 1989; *Aα3*, reported here) are shown in Figures 1, 2 and 3. Probes from one Aα4 strain, Aα4a (Table 1), were used to map the *Eco*RI fragments in other strains (Figure 1). Aα4a probes, C and D, showed no evidence of hybridization to DNA from non-Aα4 strains (Figure 1). In hybridizations to non-Aα4 DNA, probe E bound weakly with every Aα DNA after a low stringency rinse and bound only to Aα9 after a high stringency rinse. In the case of each Aα, fragment E bound to Aα fragments that are consistent with E mapping between sequences identified with probes B and F.

Aα1a probe M (Figure 2) hybridized, following low

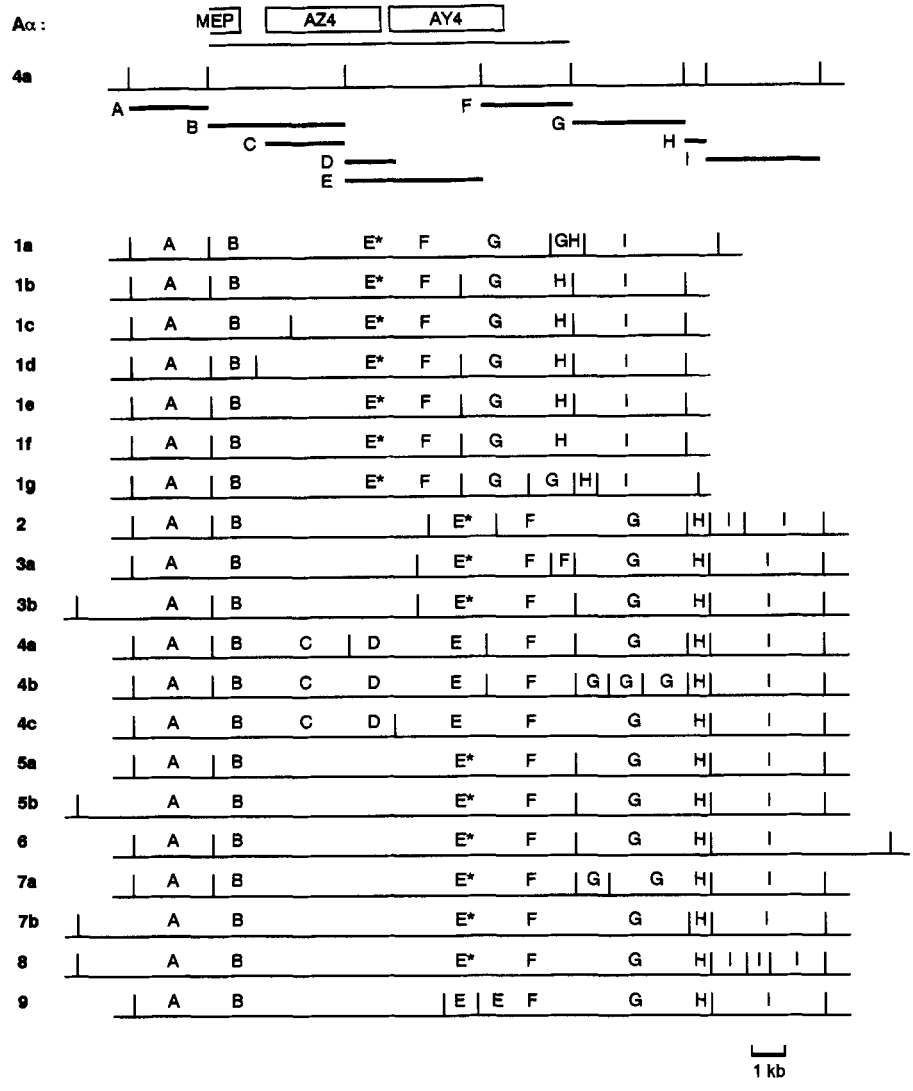

FIGURE 1.—*Eco*RI restriction maps of the *Aα* locus for 20 strains of *S. commune*. The number and letter combinations in the left column designate the Aα strain numbers as in Table 1. The map of Aα4a (at top) with *Eco*RI sites indicated by vertical lines was generated by traditional restriction enzyme mapping of the cloned cosmid DNA. Boxes indicate the coding region for a putative metalloendopeptidase (*MEP*), and Aα4a transcription regulators *AZ4*, and *AY4* (STANKIS *et al.* 1992). The other strains were mapped by a series of Southern blot hybridizations using Aα4a DNA probes to identify homologous *Eco*RI restriction fragments in genomic DNA prepared from each strain. Probes (designated A–I, top) are shown as horizontal lines below the restriction map. Aα4a probes that hybridized at high stringency to each fragment are indicated by the letter or letters assigned to that fragment. Fragments that hybridized only at low stringency with probe E are indicated by E*. Homologous *Eco*RI restriction fragments for each strain were ordered according to the position of the Aα4a probe or probes that identify them.

stringency washes, only to DNA from Aα1 strains. Aα1a probe N (Figure 2) hybridized to the same fragments from non-Aα1 strains as did Aα4a probes B and F (Figure 1). Aα1a probes J, K, and L hybridized after high stringency rinses to DNA from all seven Aα1 isolates (Aα1a–Aα1g, Figure 2).

Aα3a probe O (Figure 3) binds only to DNA from the two Aα3 isolates tested (Aα3a, Aα3b) following low stringency rinses. Aα3a probes P and Q hybridized to Aα3 DNAs after high stringency rinses (Figure 3). In both Aα3 isolates probe P identified a 0.3-kb fragment that had not been detected by Aα4a probes.

Two assumptions were made in order to assemble the maps from the hybridization data. The first assumption considered the order of restriction fragments to be conserved among *Aα* loci. The second assumption, which was required when the order of fragments was in question, considered *Eco*RI restriction sites to be conserved among loci. When the order could not be determined, *e.g.*, Aα2 and Aα8 with probe I, an arbitrary order was assigned.

The accuracy of the maps is reasonably certain. The maps of strains Aα1a, Aα3a and Aα4a determined here by hybridizations serve as controls to be compared to the maps of the cloned *Aα1a*, *Aα3a* and *Aα4a* loci generated first by standard restriction mapping methods and also from sequence data. These comparisons show no discrepancies between the data generated by these three methods. What is the likelihood of undetected fragments? For nine strains probes B and F hybridized to the same fragment, which eliminates the possibility of undetected fragments in each. In nine other strains probes B and F hybridized to different fragments; however, seven are Aα1, Aα3 and Aα4 strains, and each map has been corroborated by probing with Aα1, Aα3 and Aα4 probes, respectively (Figures 1–3). Therefore, only in strains 2 and 9 (Figure 1) is there the possibility of undetected Aα fragments.

DNA homology at the *Aα* locus: The results in Figure 1 show that there is a region of the Aα4a DNA that is unique to strains with Aα4 mating-type specificity. That region is spanned by probes C, D and E which do not

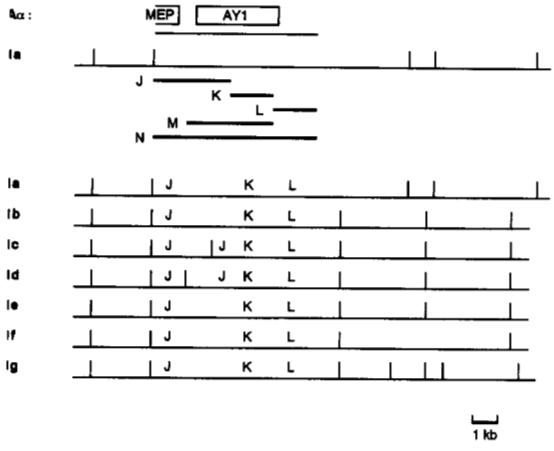

FIGURE 2.—*Eco*RI restriction maps for seven *Aα1* loci. The *Eco*RI restriction map of cloned *Aα1a* DNA (at top) with *Eco*RI sites indicated by vertical bars. Coding regions for *MEP* and, Aα1a transcription regulator *AY1*, are boxed. Maps of fragments defined by hybridizations with probes J and K are indicated with letters J and K. Results with probes M and N are discussed in the text.

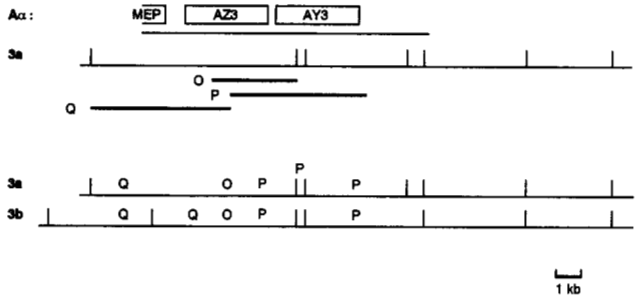

FIGURE 3.—*Eco*RI restriction maps of two *Aα3* loci. The *Eco*RI restriction map of cloned *Aα3a* DNA (at top) has *Eco*RI sites indicated by vertical bars. Coding regions for *MEP* and, Aα3a transcription regulators *AZ3* and *AY3*, are boxed. Probes O, P and Q are designated by horizontal bars. *Eco*RI restriction fragments identified by hybridization with probes O, P or Q are lettered, respectively.

hybridize to DNA from non-Aα4 strains following high stringency washes (one exception noted, probe E to Aα9). These results indicate that substantial DNA heterogeneity exists between Aα4 DNA and other Aα DNAs. Similar heterogeneities were detected when Aα1 fragment M and Aα3 fragment O were used to probe the DNAs of the other Aα specificities.

Since probe E did show weak hybridization following low stringency washes to non-Aα4 DNA, and weak hybridization to Aα9 following high stringency washes, probe E shares greater homology to DNA sequences among *Aα* loci in the region between probes D and F.

Many of the maps (Figures 1–3) have a set of restriction fragments of common size on the left, and another set of fragments of common size on the right of the heterogeneous DNA defined above. The distance separating these fragments is constant for *Aα2–Aα9* loci and almost constant for the seven *Aα1* loci. The difference

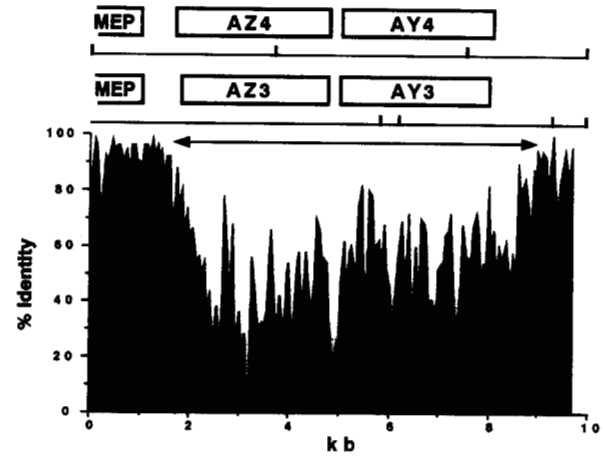

FIGURE 4.—DNA sequence comparison of Aα3 and Aα4 DNA. The genes encoding MEP, AZ4, AY4, AZ3, and AY3 proteins (boxes) are positioned above *Eco*RI restriction maps (horizontal lines with vertical bars to indicate *Eco*RI sites) of Aα4 DNA (top line) and Aα3 DNA (bottom line). The shaded graph indicates the percent identity calculated along the alignment of Aα3 DNA to 9700 bp of Aα4 DNA (*x*-axis, STANKIS *et al.* 1992; A. MARION, personal communication). A double-headed arrow estimates the length of the heterogeneous DNA found at the *Aα4* locus.

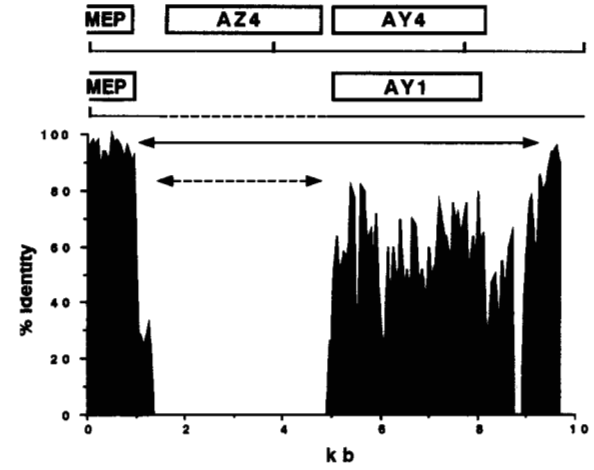

FIGURE 5.—DNA sequence comparison of Aα1 and Aα4 DNA. The genes encoding MEP, AZ4, AY4, and AY1 proteins (boxes) are positioned above *Eco*RI restriction maps (horizontal lines with vertical bars to indicate *Eco*RI sites) of Aα4 DNA (top line) and Aα1 DNA (bottom line). The shaded graph indicates the percent identity calculated along the alignment of Aα1 DNA to 9700 bp of Aα4 DNA (*x*-axis, STANKIS *et al.* 1992; A. MARION, personal communication). A double-headed arrow (solid line) estimates the length of the heterogeneous DNA found at the *Aα4* locus. A double-headed arrow (dashed line) estimates the length of DNA missing in the *Aα1* locus.

in lengths between maps of *Aα1* loci and non-*Aα1* loci is about 3.5 kb and appears to be confined to the region of DNA that is unique to each mating-type specificity.

The size of the region of heterogeneous DNA found in the *Aα* loci was more precisely estimated by sequence

comparisons of Aα1a, Aα3a and Aα4a DNAs as shown in Figures 4 and 5. Left and right boundaries indicated by arrows were arbitrarily selected where the percent identity dropped and remained below 90%. The comparison of Aα3a and Aα4a DNAs (Figure 4) shows the heterogeneous DNA in the Aα3 and Aα4 loci to be about 7.3 kb (7.2 kb for *Aα3* and 7.3 kb for *Aα4*). The comparison of Aα1a and Aα4a DNAs (Figure 5) shows the heterogeneous DNA in the *Aα1* locus to be about 4.6 kb and about 8.4 kb in the *Aα4* locus. Similar lengths of heterogeneous DNAs were defined by comparing Aα1a and Aα3a DNAs (4.6 and 8.3 kb, respectively, data not shown). The size of the heterogeneous region of *Aα1* is about 3.8 kb less than *Aα3* or *Aα4* due in part to the absence of the region that encodes Z. This difference is similar to the difference of 3.5 kb estimated from the Southern hybridization data described above.

The DNA sequence comparisons clarify the Southern hybridization results. DNA probes Aα4a: C, D, E; Aα1a: M; and Aα3a: O did not hybridize to genomic DNAs with non-self specificities following high stringency rinses. Each DNA probe has, on average, less than 60% identity to the other sequenced DNAs. Hybridization was detected following low stringency rinses only with DNA probe Aα4a: E. There are short stretches that show about 80% identity to Aα1a and Aα3a DNA. These stretches may have provided sufficient homology for detectable hybridization. Probes that hybridized to genomic DNA following high stringency rinses probably have regions of about 90% identity as found in the flanking DNA of the sequenced *Aα* loci.

DISCUSSION

PAPAZIAN (1951) in his study of strains with non-parental mating-type specificities first perceived and proved that the genetic structure of the *A* mating-type determinant was more complex than a single locus. He demonstrated that the novel (*i.e.*, non-parental) specificities were the result of genetic recombination between two linked elements he termed α and β, of the *A* factor. Subsequent studies by KOLTIN *et al.* (1967) similarly showed a bipartite structure of the *B* factor. Therefore four loci, *Aα*, *Aβ*, *Bα* and *Bβ* determine mating-type specificity in Schizophyllum. It was deduced that differences at individual loci enabled two haploid strains to be sexually compatible. We conclude from the Southern hybridization data presented here that the nine Aα specificities identified by RAPER *et al.* (1960) are defined, at least in part, by extensive differences in DNA sequence unique to each specificity in agreement with the degree of dissimilarity established between the *Aα1a*, *Aα3a* and *Aα4a* loci (STANKIS *et al.* 1992). And, as might be anticipated, isolates from different geographic locations which have the same Aα specificity have similar DNA sequences. Confirmation of our findings will require the cloning of additional *Aα*s.

Within the region of DNA sequence unique to each specificity are encoded alleles of two transcription regulators, the Z and Y proteins (STANKIS *et al.* 1992). Activation of A-regulated development requires the interaction of Z and Y proteins from *Aα* loci with different specificities (SPECHT *et al.*. 1992). Hypothetically, three types of *Aα* loci could satisfy this criterion: loci that encode different alleles of protein Z, loci that encode different alleles of protein Y, or loci that encode different alleles of both proteins Z and Y. The *Aα3* and *Aα4* loci have been shown to encode both proteins and the *Aα1* locus to encode only the Y protein (STANKIS *et al.* 1992). A comparison of the restriction maps and region of dissimilarity in DNA sequence as determined by Southern hybridizations of the 17 *Aα* loci that were not sequenced supports the following conclusions. The *Aα1* locus in general encodes only the Y protein. The *Aα2–Aα9* loci contain additional DNA and probably all encode both Z and Y proteins. A locus about the size of *Aα1* (*e.g.*, Aα1b–g in Figures 1 and 2) that does not hybridize with Aα1a probes would be indicative of a locus that encodes only the Z protein. Such an *Aα* locus was not found based on results shown in Figure 2.

The large difference in DNA sequence of different Aα mating-type specificities suggests that the evolution of different specificities was by extensive sequence divergence. Recombination between alleles of Y and Z is prohibited as a result. The absence of recombination has prevented self-activation of A-regulated development and promoted outbreeding of *S. commune*.

Other mating-type loci that encode multiple mating-type specificities (alleles) have recently been described for two other Basidiomycetes and can be contrasted to *Aα* of *S. commune*. The *b* locus of *Ustilago maydis* encodes a pair of genes: *bE* and *bW* (KRONSTAD and LEONG 1990; SCHULTZ *et al.* 1990, GILLISSEN *et al.* 1992). Different alleles of each gene, together, constitute an estimated 33 different b mating types. *b* loci that determine different b mating types, however, have undergone less sequence divergence than have the different Aα specificities of *S. commune*. Southern hybridizations cannot be used to distinguish *b* loci.

The *Aα* and *Aβ* loci determine the A mating type of *Coprinus cinereus*. In *C. cinereus* the *A* mating type loci controls clamp cell formation as *Aα* and *Aβ* do in *S. commune* (see RAPER 1966). The *Aα* locus of *C. cinereus* like the *Aα* locus of *S. commune* is about 50 kb from the *PAB1* gene found in each organism (GIASSON *et al.* 1989; MUTASA *et al.* 1990). Given the biological and genetic evidence of similarity of the *Aα* locus for each fungus it is now becoming apparent that the coding regions also have similar features. Both loci encode one or two transcriptional regulator proteins each with homeodomain motifs. The genes comparable to *Z* and *Y* in *S. commune* are respectively, *a1* and *a2* in *C. cinereus* (KUES *et al.* 1992; KUES and CASSELTON 1993). A counterpart to *Aα1*

in *S. commune* is *a2-1* of the A42 mating type in *C. cinereus*; each *Aα* locus encodes only one gene.

In *S. commune Aα* and *Aβ* are linked at 5 cM (RAPER *et al.* 1958). In *C. cinereus Aα* and *Aβ* are linked at 0.07 cM (DAY 1960). Because *Aα* and *Aβ* are functionally redundant and tightly linked, classical genetics is too difficult to determine the repetoire of different specificities for *Aα* or *Aβ* in *C. cinereus*. However, *Aα* and *Aβ* in *C. cinereus* encode dissimilar DNA such that DNAs of different specificities do not always cross-hybridize (MUTASA *et al.* 1990; MAY *et al.* 1991; KUES *et al.* 1992). DNA encoding new specificities at each locus can be identified by lack of hybridization to DNA of known specificity (MAY *et al.* 1991; KUES and CASSELTON 1993). This reinforces the importance of Southern hybridizations to readily identify different specificities at mating-type loci like *Aα* and *Aβ* of *C. cinereus* and *Aα* of *S. commune* and prepare restriction maps to aid in their cloning.

This work was supported by the Vermont Agricultural Experiment Station, University of Vermont, Burlington; and grant GM 34023 of the National Institutes of Health to R.C.U. and C.P.N. We thank AMY MARION, KIRK BARTHOLOMEW and JIAN WU for providing unpublished DNA sequence used in Figures 4 and 5.

LITERATURE CITED

CLEWELL, D., 1972 Nature of Col E_1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110:** 667–676.

DAY, P. R., 1960 The structure of the *A* mating type locus in *Coprinus lagopus*. Genetics **45:** 641–650.

GIASSON, L., C. A. SPECHT, C. MILGRIM, C. P. NOVOTNY and R. C. ULLRICH, 1989 Cloning and comparison of *Aα* mating-type alleles of the Basidiomycete *Schizophyllum commune*. Mol. Gen. Genet. **218:** 72–77.

GILLISSEN, B., J. BERGEMANN, C. SANDMANN, B. SCHOEER, M. BOLKER *et al.*, 1992 A two-component regulatory system for self/non-self recognition in *Ustilago maydis*. Cell **68:** 647–657.

HOFFMAN, R. M., and J. R. RAPER, 1974 *Schizophyllum commune*, pp. 597–626 in *Handbook of Genetics*, edited by R. C. KING. Plenum, New York.

KNIEP, H., 1920 Uber morpologische und physiologische Geschlechtsdifferenzierung (Untersuchungen an Basidiomyzeeten). Verh. Phys. Med. Ges. Wurzburg **46:** 1–18.

KOLTIN, Y., J. R. RAPER and G. SIMCHEN, 1967 Genetic structure of the incompatibility factors of *Schizophyllum commune*: the *B* factor. Proc. Natl. Acad. Sci. USA **57:** 55–66.

KRONSTAD, J. W., and S. A. LEONG, 1990 The *b* mating-type locus of *Ustilago maydis* contains variable and constant regions. Genes Dev. **4:** 1384–1395.

KUES, U., and L. A. CASSELTON, 1993 The origin of multiple mating types in mushrooms. J. Cell Sci. **104:** 227–230.

KUES, U., W. V. J. RICHARDSON, A. M. TYMON, E. S. MUTASA, B. GOTTGENS *et al.*, 1992 The combination of dissimilar alleles of the *Aα* and *Aβ* gene complexes, whose proteins contain homeodomain motifs, determines sexual development in the mushroom Coprinus cinereus. Genes Dev. **6:** 568–577.

MAY, G., L. LE CHEVANTON and P. J. PUKKILA, 1991 Molecular analysis of the *Coprinus cinereus* mating type *A* factor demonstrates an unexpectedly complex structure. Genetics **128:** 529–538.

METZENBERG, R. L., 1990 The role of similarity and difference in fungal mating. Genetics **125:** 457–462.

MUTASA, E. S., A. M. TYMON, B. GOTTGENS, F. M. MELLON, P. F. R. LITTLE *et al.*, 1990 Molecular organization of an *A* mating tupe factor of the basidiomycete fungus *Coprinus cinereus*. Curr. Genet. **18:** 223–229.

PAPAZIAN H. P., 1951 The incompatability factors and a related gene in *Schizophyllum commune*. Genetics **36:** 441–459.

RAPER, C. A., 1983 Controls for development and differentiation of the dikaryon in Basidiomycetes, pp. 195–238 In *Secondary Metabolism and Differentiation in Fungi*, edited by J. W. BENNETT and A. CEIGLER. Marcel Dekker, New York.

RAPER, C. A., 1988 *Schizophyllum commune*, a model for genetic studies of the Basidiomycotina. Adv. Plant Pathol. **6:** 511–522.

RAPER, J. R., 1966 *Genetics of Sexuality in Higher Fungi*. Ronald Press, New York.

RAPER, J. R., M. G. BAXTER and R. B. MIDDLETON, 1958 The genetic structure of the incompatibility factors in *Schizophyllum commune*. Proc. Natl. Acad. Sci. USA **44:** 889–900.

RAPER, J. R., M. G. BAXTER and A. H. ELLINGBOE, 1960 The genetic structure of the incompatibility factors of *Schizophyllum commune*: the *A* factor. Proc Natl. Acad. Sci USA **44:** 889–900.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SCHULZ, B., F. BANUETT, M. DAHL, R. SCHLESINGER, W. SCHAFER *et al.*, 1990 The *b* alleles of *U. maydis*, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. Cell **60:** 295–306.

SOUTHERN, E., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

SPECHT, C. A., C. C. DIRUSSO, C. P. NOVOTNY and R. C. ULLRICH, 1982 A method for extracting high-molecular-weight deoxyribonucleic acid from fungi. Anal. Biochem. **119:** 158–163.

SPECHT, C. A., M. M. STANKIS, L. GIASSON, C. P. NOVOTNY and R. C. ULLRICH, 1992 Functional analysis of the homeodomain-related proteins of the *Aα locus of Schizophyllum commune*. Proc. Natl. Acad. Sci. USA **89:** 7174–7178.

STANKIS, M. M., C. A. SPECHT, H. YANG, L. GIASSON, R. C. ULLRICH *et al.*, 1992 The *Aα* mating locus of *Schizophyllum commune* encodes two dissimilar, multiallelic, homeodomain proteins. Proc. Natl. Acad. Sci. USA **89:** 7169–7173.

Communicating editor: P. J. PUKKILA