The combination of dissimilar alleles of the $A_{\alpha}$ and $A_{\beta}$ gene complexes, whose proteins contain homeo domain motifs, determines sexual development in the mushroom *Coprinus cinereus*

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The $A$ mating-type factor is one of two gene complexes that allows mating cells of the mushroom *Coprinus cinereus* to recognize self from nonself and to regulate a pathway of sexual development that leads to meiosis and sporulation. We have identified seven $A$ genes separated into two subcomplexes corresponding to the classical $A_{\alpha}$ and $A_{\beta}$ loci. Four genes, one $\alpha$ and three $\beta$, all coding for proteins with a homeo domain-related motif, determine $A$-factor specificity; their allelic forms are so different in sequence that they do not cross-hybridize. It requires only one of these four genes to be heteroallelic in a cell to trigger $A$-regulated sexual development, and it is the different combinations of their alleles that generate the multiple $A$ factors found in nature. The other three genes cause no change in cell morphology and may regulate the activity of the four specificity genes.

[Key Words: Mating type; *Coprinus*; sexual development; sexual compatibility; homeo domain motifs]

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The mushroom *Coprinus cinereus* has a typical hymenomycete life cycle with two functionally distinct mycelial stages. A single sexual spore germinates to give a uninucleate-celled mycelium, the monokaryon, which is sexually sterile but produces abundant asexual spores, the oidia. For sexual reproduction, two compatible monokaryons must mate to give the fertile binucleate-celled mycelium, the dikaryon, which no longer produces oidia, but given the right temperature and light conditions, differentiates the fruiting bodies. Hyphal fusion is sufficient for mating and initiates the developmental pathway outlined in Figure 1. Nuclei are exchanged, and the donor nucleus in each case migrates through the established cells of the recipient hyphae, triggering the breakdown of cell septa until it reaches the hyphal tip cell. A complex mode of cell division then occurs that involves formation of a specialized structure at each septum, the clamp connection, through which one nucleus must pass. The clamp ensures the equal distribution of both genetically different nuclei to each daughter cell, because it is these two nuclei that will eventually fuse and undergo meiosis in special cells on the undersurface of the mushroom (Casselton 1978).

The genes that determine monokaryon compatibility and regulate sexual development are the mating-type genes. Two species have been used as experimental models to study hymenomycete mating-type gene function, *C. cinereus* (Casselton 1978) and *Schizophyllum commune* (Raper 1983). From classic genetics it was established that in both fungi there were two unlinked genetic determinants that were called the $A$ and $B$ mating-type factors [see Raper 1966]. These have multiple specificities (designated $A_1$, $A_2$, $A_3$, etc., and $B_1$, $B_2$, $B_3$, etc.) that must be different for dikaryon formation. As indicated in Figure 1, $A$ and $B$ independently regulate different steps in dikaryon morphogenesis; the $A$ factor described in this paper governs the synchronized division of the mated nuclei and the formation of the clamp cell, whereas clamp cell fusion and nuclear migration require different $B$ factors. There is no barrier to fusion of incompatible hyphae, and when only one factor is different, this leads to the isolated operation of the part of the sequence it regulates. When only $A$ is different, unfused clamp cells develop (Sweizynski and Day 1960). In *C. cinereus* and *S. commune*, it was shown by classic genetics that the $A$ factor derives its multiple specificities from two closely linked multiallelic genes termed $\alpha$ and $\beta$. 

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new regulatory protein from polypeptides coded by heteroalleles of these genes.

Results

Transcript analysis identifies seven A-factor genes

In our initial analysis of the cloned A42 factor, we identified two genes that were both able to elicit A-regulated sexual development (formation of unfused clamp cells, Fig. 1) when introduced into host cells with different A factors. Because we had shown that 0.1 map units represent a physical distance of 10 kb [Mutasa et al. 1990], we expected the α and β loci to be ~7.0 kb apart, which would correspond to the 0.07 map units determined by recombination analysis [Day 1960]. The two genes identified were unexpectedly close together, however, and only 1.0 kb apart. A particularly puzzling feature was that the genes were embedded in 9.0 kb of A42-specific DNA sequence that was shown to code for three different transcripts [Mutasa et al. 1990]. These two facts suggested that the A factor might be a larger complex of genes than predicted by genetic analysis and that we had identified only one of the A loci.

To look for other A genes, a detailed restriction map of the DNA sequence containing A42 was constructed. Starting from the position of the genes identified previously, a sequence of 40 kb was examined for transcripts. A series of probes was chosen to cover this region and used to identify A42 factor-specific mRNAs in Northern blots of poly(A) RNA isolated from an A42 monokaryon. Two discrete clusters of genes, one with two transcripts and one with five transcripts, were identified, which were both shown by transformation to promote clamp cell development [see below]. These clusters were separated by a 7.0-kb sequence from which no transcripts could be detected (Fig. 2)—a distance corresponding exactly to that predicted to separate the α and β loci (see above).

The A factor is flanked by two closely linked genes pab-1 and ade-8 [Day 1960]; the A42 factor was isolated by a chromosome walk from pab-1. By correlating the genetic map with the physical map shown in Figure 2, we can distinguish the two-gene cluster closer to pab-1 as the α locus and the five-gene cluster as the β locus (Fig. 3). The two α genes, designated a1 and a2, have transcripts of 2.6 and 2.1 kb, respectively, and are separated by 1.0 kb of noncoding sequence. At the β locus, β1, β2, and β3 with transcripts of 2.5, 2.2, and 2.45 kb, respectively, are the three genes identified by Mutasa et al. [1990]. β1 and β2 are separated by 1.0 kb of noncoding sequence from β3. β4 has a 2.5-kb transcript and β5 a smaller and relatively more abundant 1.1-kb transcript. The direction of transcription in each case was determined by selective strand labeling of probes and confirmed by sequencing the 3’ ends of cloned cDNAs [for details, see Materials and methods]. β1 and β5 are transcribed in the opposite direction to the other five genes; the β4 and β5 transcripts overlap by 25 bases at their 3’ ends. Because the A42 genes are the first A-factor genes

Figure 1. Regulation of dikaryon formation by the A and B mating-type genes of the mushroom C. cinereus.
### Figure 2. Physical map and corresponding transcript analysis of the A42 mating-type region. Restriction sites relevant to this analysis are abbreviated as follows: (B) BamHI; (C) Clal; (E) EcoRI; (H) HindIII; (P) PstI; (S) Sall. The chromosomal positions of the fragments used as hybridization probes in Northern blots (either subcloned in pBluescript or gel-purified fragments; see Materials and methods) are shown below the map. Numbers refer to the corresponding autoradiographs that show the transcripts detected by each of these probes. The arrows give the sizes and positions of transcripts with reference to the physical map and direction of transcription defined by selective strand labeling of pBluescript subclones. (10', 10'') An example of using labeled single strands of plasmids pESM6 and pUK6 to differentiate two overlapping transcripts. The relative positions of the two clusters of transcripts identified as the α and β complexes are indicated above the map.

of \( C. \) cinereus characterized at the molecular level, we have given each gene the allele designation 1 (i.e., \( a1-1, a2-1 \), etc., in Fig. 3).

**The genes of the A factor share no detectable sequence similarity**

The lack of functional differentiation between \( \alpha \) and \( \beta \), shown by classic studies, suggested that one locus might be a duplication of the other [Raper 1966]. Although we now show that there are more than two genes, the same argument could apply because of the obvious similarities in transcript sizes. If the genes have a common evolution we could expect to detect some sequence similarity and accordingly have looked for cross-hybridization. Five of the A42 genes have been cloned separately as Sall fragments [pUK1-5; Fig. 4]; a sixth Sall fragment cloned in pUK6 contains the overlapping \( \beta4 \) and \( \beta5 \) genes. Each of the plasmids was used separately as hybridization probe against Sall-cut pUK plasmids [Fig. 4A]. By cutting pUK6 with Sall and EcoRI, \( \beta4-1 \) and \( \beta5-1 \) were separated as close to the point of overlap as possible, and the fragments generated were tested for cross-hybridization in a separate experiment [Fig. 4B].

We could detect no cross-hybridization between any of the genes, suggesting that they are very different in nucleotide sequence.

**A mating-type specificity can be determined by four genes**

The introduction of a compatible A factor into a haploid host by transformation has the same effect as a compatible mating; it elicits A-regulated clamp cell development [Mutasa et al. 1990; May et al. 1991]. Each of the A42 genes was tested separately to see whether it was sufficient to activate A-regulated development in five different A-factor backgrounds, A42 [control], A3, A5,
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Figure 3. Correlation of the genetic map, based on recombination data of Day (1960), and the physical map derived from this study. \( \alpha \)-1, \( \beta \)-1, \( \beta \)-2, and \( \beta \)-4 (stippled boxes) are the A-factor specificity genes that have alleles with unique sequence and that promote A-regulated sexual development when introduced by transformation into a host cell with a different allele of the gene. \( \beta \)-3-1 (open box) is a nonspecificity gene with unique allele sequences, \( \alpha \)-1 and \( \beta \)-5-1 (solid boxes) are nonspecificity genes that have common sequences in all A factors tested (Fig. 6; Table 1). Sizes of transcripts and direction of transcription (Fig. 2) are indicated below the genes.

A6, and A43. No genetic data were available to indicate whether any of these factors share the same \( \alpha \) or \( \beta \) gene complex; Day's (1963) analysis showed only that A5 and A6 have different \( \alpha \) and \( \beta \) alleles.

To select transformants, a cotransformation system based on tryptophan auxotrophy was used. Cotransformation occurs with 30–60% efficiency in C. cinereus (Mellon et al. 1987; Casselton and de la Fuente Herce 1989), but not all transforming DNA is expressed (Mellon and Casselton 1988). Trp\(^{+}\) transformants were screened for clamp cell development. Where a negative result was recorded, 50–100 transformants were checked. The results of these tests are summarized in Table 1. Four genes, \( \alpha \)-2, \( \beta \)-1, \( \beta \)-2, and \( \beta \)-4, individually elicited A-regulated clamp cell development when introduced into two hosts having either the A5 factor or the A3 factor. The obvious morphologic change caused by introduction of these four A42 genes into an A5 host is illustrated in Figure 5. \( \alpha \)-2-1 did not promote clamp cell development in an A6 host, but did in the A43 host. \( \beta \)-4-1 failed to induce clamp cell development in both the A6 and the A43 hosts. As expected, none of the A42 genes elicited clamp cell development when introduced into the A42 host. The \( \alpha \)-1-1, \( \beta \)-3-1, and \( \beta \)-5-1 genes caused no detectable phenotypic change in any of the hosts. Because we know the precise location of the 3' ends of the transcripts of each gene, we are confident that the fragments used for transformation (SalI fragments of 4.2, 3.55, and 4.8 kb) are large enough to contain the entire coding sequence together with ~500 bp of 5'-flanking sequence. This transformation analysis has allowed us to distinguish at least two functionally distinct classes of genes in the A factor. The genes that promote clamp cell development clearly determine A-factor specificity, and we now refer to these as the specificity genes.

Figure 4. Cross-hybridization analysis of A42 factor genes. (A) Plasmids pUK1-6 were cut with SalI to release the subcloned genes, and each plasmid was then used individually as probe to the same Southern filter. \( \beta \)-1, \( \beta \)-2, etc., refer to the respective genes present in each lane and to the corresponding autoradiograph obtained using the cloned gene as hybridization probe. (B) pUK6 cut with SalI and EcoRI to release \( \beta \)-4 and \( \beta \)-5 on separate fragments and corresponding hybridization data.

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The A factors we have tested share the same α or β alleles (Day 1963), but our hybridization and transformation data indicate that A6 and A42 share the same α specificity. These studies demonstrate that these two factors can also share alleles of a gene within the β complex and still have a different overall specificity.

There are two genes, one in each cluster, that appear to be the same in all A factors, α1 and β5. As far as we can detect, there are no differences in the level of cross-hybridization between α1 genes in the different DNAs. For β5, the corresponding gene in the A5 factor was barely detectable, suggesting that there is some sequence variation among its alleles. We have, however, distinguished two classes of genes by this analysis: unique sequence genes (α2, β1, β2, β3, and β4) and common sequence genes (α1 and β5).

The 7-kb region that separates the α and β gene complexes (see Fig. 2) was shown to be homologous in all strains (Fig. 6), as expected by the reciprocal recombination that occurs between α and β (Day 1960). We have called this region the homologous hole of the A factor. It

### Table 1. Identification of the four A42-factor specificity genes that promote A-regulated clamp cell development by DNA-mediated transformation into hosts with different A factors

| Host A factor | α-Complex | β-Complex |
|---------------|-----------|-----------|
| A3            | a1-1      | b1-1      |
| A5            | a2-1      | b2-1      |
| A6            | -         | b3-1      |
| A43           | -         | b4-1      |
| A42           | -         | b5-1      |

Clamp cell development identifies a specificity gene (+); (-) failure of the specificity gene to promote clamp cells in this host resulting from the fact that the host A factor has the same allele of the gene. [−] The gene did not promote clamp cell development in any host.

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### Figure 5. A-regulated clamp cell development elicited in an A5 host cell by transformation with A42 specificity genes. The untransformed A5 monokaryon has only simple septa (top). Transformants with α2-1, β1-1, β2-1, and β4-1 (below) all produce unfused clamp cells at each septum.
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Figure 6. Genomic analysis of five different A factors. cDNAs corresponding to each of the A42 genes were used to probe HindIII-cut genomic DNA (5 µg) of monokaryons having the A factors indicated for each lane on the autoradiographs illustrated. Size markers [in kb] indicate the HindIII fragments of the A42 factor. The respective hybridization pattern obtained for each probe is indicated by the gene designation above the autoradiograph. The results of using two different probes are illustrated for β1, (β1 and 3′β1) to show the common 3′ sequence in all of its alleles. The noncoding sequence between the a and β complexes was investigated using two genomic HindIII fragments of 4.3 and 1.9 kb cloned in pAMT6 and pAMT7. The two HindIII-BamHI fragments of pAMT7 (see physical map in Fig. 2) gave the same hybridization pattern as the whole plasmid [panel 3, top] confirming a length of ~7.0 kb of homologous DNA constituting the homologous hole.

is homologous because all A factors share this DNA sequence and a hole, from the point of view that we cannot detect any transcripts from it.

The A-factor specificity gene proteins contain a homeo domain-related motif

Although the complete sequences of the four A42 specificity genes are not yet available, preliminary data show that all encode proteins containing a putative DNA-binding domain, a homeo domain-related motif [Scott et al. 1989]. The sequences of these domains have been compared with those present in the a1 and a2 mating-type proteins of Saccharomyces cerevisiae (Fig. 7). In the a2-1 and β2-1 proteins, the homeo domain is more similar to that of a1, and in the β1-1 and β4-1 proteins it is more similar to that of a2.

Discussion

The genes we have described constitute an A mating-type factor of the mushroom C. cinereus, a complex that determines compatibility in mating by regulating a developmental sequence that leads to fruit body development, meiosis, and sporulation. We have identified seven genes separated by 7 kb of noncoding homologous DNA sequence into two subcomplexes that correspond to the a and β loci identified by classic recombination analysis. The organization of these genes is summarized in Figure 3. We have used sequence similarity and function as criteria for characterizing the A genes, and this has enabled us to distinguish the three classes represented by the different boxes in Figure 3. Four genes, one a and three β [stippled boxes], determine A-factor specificity. The alleles of each gene have unique sequences. It is only when the same allele is present in two different A factors that we can detect cross-hybridization [Fig. 6]. Transformation data suggest that it requires only one of these genes to be made heteroallelic in a cell to promote A-regulated sexual development [i.e., a2-1, not present in A3, A5, or A43, triggers clamp cell development when introduced into host cells with any one of these A factors]. A third gene with unique sequence alleles [open
fungal mating-type proteins as transcription factors has been elegantly demonstrated in (for review, Schulz et al. 1990). Preliminary sequence data show that these encode putative DNA-binding proteins, each with a homeo domain-related motif (Fig. 7), which suggests that they are transcription factors. The role of fungal mating-type proteins as transcription factors has been elegantly demonstrated in S. cerevisiae (for review, see Herskowitz 1989), and increasing evidence suggests that this is also true in other species. The three polypeptides coded by the S. cerevisiae a1, a1, and a2 genes all bind DNA sequences upstream of regulated genes to activate or repress a- and a-specific haploid cell functions or as an a1–a2 dimer to repress haploid functions in a diploid cell (see Herskowitz 1989). It is particularly relevant that a1 and a2 polypeptides have a homeo domain-related motif (Kelly et al. 1988). A similar motif is found in the mat1–P1 mating-type protein of Schizosaccharomyces pombe (Kelly et al. 1988) and in the multiallelic b gene polypeptides of Ustilago maydis (Schulz et al. 1990). Preliminary sequence data for Aα gene alleles of S. commune show that these alleles also encode proteins with homeo domain-related motifs (Ullrich et al. 1991).

Transcript analysis has enabled us to identify three other genes associated with the specificity genes. The tight clusters that these genes form strongly suggest that they are part of the A-factor complex. a1 and bβ are the flanking genes and have a DNA sequence that appears to be at least partly homologous in the different A factors examined, but there is no homologous flanking sequence between these genes and the adjacent specificity genes a2 and b4. b4 and b5 are transcribed in opposite directions with overlapping 3′ ends, and several long transcripts of a1, only identified by cDNA cloning (Kues, unpbl.), overlap the 5′ end of the a2 gene. The b3 gene is particularly puzzling: As with the specificity genes, its alleles lack any detectable homology, but heteroallelism of this gene alone is insufficient to promote A-regulated sexual development. With so many specificity genes and their apparent redundancy, it is possible that some of their alleles are nonfunctional. We do not know, as yet, the function of the other two genes. It is tempting to speculate that they may have a role in post-translational activation, as we have been unable to detect any developmental regulation of A-gene transcription.

**Figure 7.** Comparison of homeo domain-related motifs of A42 specificity gene proteins of C. cinereus and those of the a1 and a2 mating-type proteins of S. cerevisiae (Shepherd et al. 1984). The most invariant amino acids in the homeo domain, WF-N-R, which are found in the recognition helix (Scott et al. 1989), are marked by asterisks (*).

**Function of the A-factor genes**

A compatible mating requires that the A factors of the two mating cells have different specificities. We have shown by DNA-mediated transformation that there are four genes in the A42 factor that determine this specificity, a1, b1, b2, and b4. Preliminary sequence data show that these encode putative DNA-binding proteins, each with a homeo domain-related motif (Fig. 7), which suggests that they are transcription factors. The role of fungal mating-type proteins as transcription factors has been elegantly demonstrated in S. cerevisiae (for review, see Herskowitz 1989), and increasing evidence suggests that this is also true in other species. The three polypeptides coded by the S. cerevisiae a1, a1, and a2 genes all bind DNA sequences upstream of regulated genes to activate or repress a- and a-specific haploid cell functions or as an a1–a2 dimer to repress haploid functions in a diploid cell (see Herskowitz 1989). It is particularly relevant that a1 and a2 polypeptides have a homeo domain-related motif (Kelly et al. 1988). A similar motif is found in the mat1–P1 mating-type protein of Schizosaccharomyces pombe (Kelly et al. 1988) and in the multiallelic b gene polypeptides of Ustilago maydis (Schulz et al. 1990). Preliminary sequence data for Aα gene alleles of S. commune show that these alleles also encode proteins with homeo domain-related motifs (Ullrich et al. 1991).

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**Determination of A-factor specificity**

This study shows that it requires only one of the four A-factor specificity genes to be made heteroallelic for A-regulated sexual development to proceed. We conclude that nonself recognition and developmental regulation are jointly governed by the ability of a cell to synthesize polypeptides coded by heteroalleles of these genes. It is then logical to suggest that heteroallelic polypeptides can associate to form novel regulatory proteins not present in unmated cells. This idea is similar to models proposed by Schulz et al. (1990) to explain the recognition function of the b gene of the hemiasdiaspymycete smut fungus U. maydis. A precedent for the interaction of different mating-type polypeptides is provided by S. cerevisiae, where the a1–a2 dimer is a new regulatory protein that can only be formed in mated cells (see Herskowitz 1989, Dranginis 1990). It may be significant that a1 and a2 have dissimilar homeo domains (Shepherd et al. 1984). Two of the homeo domains in the C. cinereus A42 mating-type proteins (a2-1 and b2-1) are more similar to that of a1, and two (b1-1 and b4-1) are more similar to that of a2 (Fig. 7). This could suggest that the interaction that triggers sexual development in C. cinereus is between nonallelic homeo domain proteins. Our transformation experiments do not rule out this possibility because of the large number of specificity genes present in each factor.

The problem of polypeptide recognition in basidiomycetes is more complex than in S. cerevisiae because there are multiple alleles, not just two alleles at the mating-type locus. In U. maydis there are ~25 alleles of the b gene, which controls both mating compatibility and formation of the pathogenic dikaryon (Rowell and DeVay 1954, Puhalla 1968). These alleles are largely ho-
mologous in sequence, and alleles encode polypeptides with only a variable amino-terminal domain (Kronstad and Leong 1990; Schulz et al. 1990). In both hymenomycetes studied, C. cinereus shown here and S. commune (Giasson et al. 1989), alleles of the A mating-type genes do not cross-hybridize, indicating very different nucleotide sequences. This does not preclude some similarity at the protein level, as shown by preliminary sequence data for alleles of the same mating-type gene of S. commune (Ullrich et al. 1991). Allelic genes are different enough not to cross-hybridize, but their proteins may still have conserved regions necessary for polypeptide recognition. This leads to an important problem, highlighted in our study. With four specificity genes, apparently identical in function, how are polypeptides brought together by mating distinguished from those already present in the unmated cell? It seems likely that some of the specificity genes are redundant. It will require us to generate null mutants to demonstrate this, a difficult task in C. cinereus because homologous gene replacement by transforming DNA is extremely rare (Binninger et al. 1991).

Multiple A-factor specificities

Day [1963] identified 31 A factors in a sample of 33 independent isolates of C. cinereus. From this analysis it is predicted that >160 A factors exist [Raper 1966]. We have shown that four genes determine specificity and that different A factors can share their alleles. If all allele combinations are possible, only four alleles of each gene are required to generate 256 A factors. It is unlikely, however, that these genes are easily reassembled in nature. Separation of the a2 gene into a separate subcomplex allows this to be recombined with all specificity genes in the β complex, but the organization within the β complex suggests that its genes are rarely recombined. Not only do alleles of the genes lack sequence similarity, but flanking sequences are also nonhomologous [Mutasa et al. 1990]. Reciprocal recombination is possible only if a region of homology is presented by a shared a1 allele. The only shared alleles detected in this study were β4-1 and β5-1. Because of their positions at the border of the complex, recombination within these genes would not recombine the other specificity genes to generate new A factors. Because of the close linkage of α and β, the only estimates of allele numbers at each locus are from a small survey of 10 A factors studied by Day [1963], which identified four α and five to seven β alleles. Unfortunately, only two of the characterized factors (A5 and A6) are still available. Day's studies lead us to expect at least four alleles of the a2 specificity gene. A molecular approach will be required to determine whether the three β specificity genes are also multiallelic. The A43 factor of C. cinereus used in this study has been cloned by May et al. [1991]. The investigators provide evidence for three specificity genes in this factor. We have shown here that A43 and A42 share the allele of one of these (β4-1) [Fig. 6]. Better resolution of A43 by transformation analysis has revealed one specificity gene at the α locus and two genes in addition to β4-1 at the β locus [U. Kues et al., unpubl.]. As expected from the hybridization data [Fig. 6], all of these alleles are different from those present in A42. Because both sets of specificity genes trigger A-regulated development in an A3 host, we can conclude that at least two of the β specificity genes are multiallelic.

This study shows that the A mating-type factor is far more complex than predicted by classic genetics. With four different genes, all contributing a unique specificity, our analysis provides an insight into how such large numbers of different A factors have developed in nature.

Materials and methods

C. cinereus strains include A42B42: JV6 wild type; LN118 ade-2 trp-1.1,1.6; A6B6: H9 wild type; LT2 trp-1.1,1.6; A5B6: H5 wild type; FA2222 trp-1.1,1.6; A3B1: C692 ade-3; 218 trp-1.1,1.6; A43B43: OK130 ade-8; AT8 ade-8 trp-1.1,1.6. Media and methods for culturing C. cinereus have been described by Lewis [1961], with modifications summarized by Mutasa et al. [1990]. The transformation procedure was based on that of Binninger et al. [1987], as modified by Casselton and de la Fuente Herce [1989]. Plasmid pCc1001, containing the C. cinereus trp-1 gene [Binninger et al. 1987], and pDB1, containing the C. cinereus trp-3 gene [D.M. Burrows, T.J. Elliott, and L.A. Casselton, unpubl.], were used for cotransformation experiments with trp auxotrophic C. cinereus strains. Photographs of clamp cells were taken with a Zeiss photomicroscope using phase contrast.

DNA manipulations

Genomic DNAs of C. cinereus strains were prepared either by the method of Wu et al. [1983] or by the small-scale method of Zolan and Pukkila [1986]. Routine cloning and plasmid amplification was in Escherichia coli strains XL-1 Blue [recA1, lac-, endA1, gyrA96, thi, hsdR17, supE44, relA1, F proAB, lacIq, lacZAM15, Tn10] [Stratagene] or DH5α [F, endA1, hsdR17 (m-, m-)’, supE44, thi-1, recA1, gyrA96, relA1, 6800ΔlacZM15] (BRL) using standard DNA techniques (Sambrook et al. 1989).

Plasmids constructs with C. cinereus DNA

pEC1, pEC12, and pEC16 are recombinant clones from a cosmid library constructed in Lorist2 and contain parts or the whole sequence of the A42 mating-type factor [Mutasa et al. 1990]. Gel-purified fragments or restriction digests of these cosmids clones were subcloned into the polylinker of either pBluescript (KS−) or pUC13 (pBG-series) and used for restriction and hybridization analysis to map the A42 α and β loci and to complete the restriction map given earlier [Mutasa et al. 1990]. [Compare Fig. 2 to localize subcloned fragments especially mentioned for transformation, hybridization, and sequencing experiments.]

pBluescript (KS−) subclones of genomic fragments used for Coprinus transformations pAMT1 contains β1-1 on a 3.0-kb HindIII fragment, and pESM1 contains β2-1 on a 2.8-BamHI–SalI fragment [Mutasa et al. 1990]. pUK1, pUK2, and pUK4–pUK6 have genes α1-1, α2-1, β2-1, β3-1, and β5-1 on SalI fragments of lengths 4.2, 2.1, 3.4, 3.55, and 4.8 kb, respectively. pUK6 contains β5-1, together with an inactive truncated copy of β3-1 on a 4.8-kb SalI fragment. pESM2 contains the complete β4-1 gene on a 4.0-kb BamHI fragment.
Subclones used for Northern analyses  pAMT1, pUK1, pESM2, and the following plasmid subclones were used for Northern analyses. pUK7 contains a 2.9-kb EcoRI–HindIII fragment containing most of the α2-1 gene. pAMT6 and pAMT7 contain 4.0- and 1.9-kb HindIII fragments, respectively, which identify the noncoding sequence between the α and β genes. pUK3 has a 3.5-kb Sall fragment containing the β1-1 gene. A 0.5-kb HindIII and a 0.4-kb HindIII–EcoRI fragment with the 5′ end of gene β2-1 was subcloned after partial restriction of pCE12 to give pBG1. pBG2 contains a 2.9-kb EcoRI–HindIII fragment with the 3′ end of gene β2-1, and pBG3 contains a 2.5-kb HindIII–EcoRI fragment with most of the β3-1 gene sequence. pESM3 is a subclone of pBG2 containing a 1.2-kb PstI–Sall fragment. pESM4, pESM5, pESM6, and pUK8 carry a 1.3-kb BamHI–HindIII fragment, a 1.0-kb HindIII fragment, a 1.7-kb HindIII–BamHI fragment, and a 0.7-kb HindIII–EcoRI fragment, respectively, each with parts of the β4-1 gene sequence. pUK9 has a 1.0-kb EcoRI–BamHI fragment carrying part of β5-1.

pLAC1 was isolated from a plasmid genomic library of C. cinereus strain H9 constructed by Pukkila et al. (1984), using the A42 α2-1 gene as hybridization probe. pLAC1 contains the two genes of the A6 α locus. Because A6 and A42 have the same alleles of these two genes, fragments from pLAC1 subcloned into pBluescript(ks−) could be used as gene probes in Northern hybridizations. pHH5 and pHH7 contain the 5′ and 3′ ends of the α1-1 gene cloned on a 2.4-kb and 1.1-kb EcoRI fragment, respectively. pUK10 contains part of the α2-1 gene on a 2.0-kb Clal–BamHI fragment.

Subclones used for Southern analysis  pUK1–pUK6 were used to detect homology between different A42 genes. cDNAs for all A-α gene transcripts were subcloned from αgt10 into pBluescript(ks−) with either the EcoRI or the BamHI sites of the αgt10 adapter. These were used to detect homooligalic genes in genomic DNA digests. The length of the homologous holc was defined using gel-clutered fragments of pAMT6 and pAMT7.

RNA isolation  Strain IV6 was grown in liquid minimal medium, and total RNA was prepared using the guanidinium thiocyanate–CsCl step gradient procedure (Glisn et al. 1974; Sambrook et al. 1989), with the modification suggested by Chirgwin et al. (1979) of resuspending the pellet in 7.5 M guanidinium hydrochloride after the ultracentrifugation step. Standard extractions were of resuspending the pellet in 7.5 M guanidinium hydrochloride.

DNA polymerase I-generated using the Klenow fragment of E. coli.

Nucleic acid blots and hybridizations  Gels prepared for Southern or Northern analyses were blotted onto Hybond-N membranes (Amersham) in 20× SSC (3 M NaCl, 0.3 M sodium citrate). For Southern blots, hybridizations were performed overnight at 65°C as described by Mellon et al. (1987). Hybridizations of Northern blots and washes were performed as recommended for Hybond-N by the manufacturers. For autoradiography, filters were exposed to Fuji X-ray film. Filters for Southern and Northern analyses were stripped (see manufacturers’ instructions) and reused to allow direct size comparisons.

Gels isolated DNA fragments or plasmid clones for Southern blots were labeled with [α-32P]CTP using a nick-translation kit (BRL). High-activity hybridization probes for RNA blots were generated by labeling double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA) with [α-32P]CTP (>3000 Ci/mmole, Amersham) using a random primer kit (Boehringer). dsDNA probes used for Northern analyses, indicated in Figure 2 but not described above, were gel-purified restriction fragments of the cosmids or pUC13 and pBluescript(ks−) subclones. To determine transcript directions, single-stranded DNAs were prepared from pBluescript(ks−) subclones using the helper phage M13K07 (Sambrook et al. 1989), and labeled strands were generated using the Klenow fragment of E. coli DNA polymerase I and the M13 reverse primer (Ley et al. 1982; Sambrook et al. 1989).

Construction of a cDNA library  A αgt10 cDNA library was constructed with mRNA isolated from wild-type strain IV6 and the cDNA synthesis system plus and cDNA cloning system–αgt10 from Amersham (RPN 1256 and RPN 1257) using the recommended E. coli strain NM514 (hsdR514 [fr- mK] argB, galE, galK, Str−, Lac− [HI−]) as host. The library was screened with pBluescript(ks−) subclones. Screening of 10^6 to 10^7 plaques was needed to detect one copy of each gene.

Sequencing  All sequencing was performed with dsDNA of pBluescript(ks−) or pUC13 subclones, 5'-labeled ATP, the T7 polymerase kit of Pharmacia, and either the M13 universal or reverse primers. Homeo domain motifs (Fig. 7) were deduced from partial sequence data of cDNA clones. Transcription directions of all genes were confirmed by sequence analysis. cDNA 3′ ends were located within specific genomic fragments identified by the transcript mapping analysis. The results of these analyses can be summarized with respect to the gene, the plasmid construct, sequence, and relative position on the restriction map shown in Figure 2: α1-1, pHH7, TCAGAATCCAGTTTCT-ACGGG, 0.25 kb upstream of the BamHI site; α2-1, pAMT6aEcoRI, TTGCATTTCA'AGCACATTTC, 0.12 kb upstream of the EcoRI site; β1-1, pAMT1, ATCCAGGCATACTTGCGA, 0.3 kb upstream of the HindIII site; β2-1, pESM1, CCAGTCGCTA'AGTCACATTTC, 0.2 kb downstream of the PstI site; β3-1, pBG3, TCTGCGCTCAGGATGGCAA, 0.5 kb upstream of the EcoRI site; β4-1 and β5-1, pUK8 CAATACAGACAGACAGTTTCTCTTAGGACAT, 0.05 kb from the EcoRI site. The asterisk in each case indicates the polyadenylation site. * indicates the position of the αα gene transcript in the opposite DNA strand, * indicates the end of the β4-1 transcript.

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The combination of dissimilar alleles of the A alpha and A beta gene complexes, whose proteins contain homeo domain motifs, determines sexual development in the mushroom Coprinus cinereus.

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