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Genetics

Identification of genes governing filamentous growth and tumor induction by the plant pathogen *Ustilago maydis*

(mating type loci/regulatory gene targets/repression/fungal pathogen of corn)

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ABSTRACT **Two master regulatory loci, *a* and *b*, govern life-cycle transitions of the phytopathogenic fungus *Ustilago maydis*. Fusion of haploids that differ at both *a* and *b* results in production of a filamentous dikaryon, which induces tumors in its host, maize. Here I describe identification of genes distinct from *a* and *b* that play roles in these life-cycle transitions. These studies identify three genes, *fuz1*, *fuz2*, and *rtf1*, that are necessary for filament formation. *fuz1* is also necessary for normal size and distribution of tumors and for teliospore formation; *fuz2* is also necessary for teliospore germination. Mutations in the *rtf1* gene, which are recessive, bypass the requirement of different *b* alleles for tumor formation. This observation indicates that *rtf1* codes for a negative regulator of tumor induction. The *fuz1*, *fuz2*, and *rtf1* genes may be targets for the *a* and *b* loci.**

Ustilago maydis, a basidiomycetous fungus that induces tumors on corn plants, is characterized by its dimorphism: haploid cells exhibit yeast-like growth on a variety of laboratory media and are not pathogenic, whereas dikaryons (the product of mating of two compatible haploids—i.e., having different *a* and *b* alleles) are filamentous, grow poorly if at all on laboratory media, and are pathogenic. The filamentous dikaryon differentiates within tumors, where karyogamy and spore formation occur and where these spores (teliospores) acquire competence to undergo meiosis (1, 2). The developmental program responsible for producing these two forms and for completing the above steps in the life cycle is governed by two master regulatory loci, *a* and *b* (the mating type loci), known from classical genetic studies (3–6).

The *b* locus, with 25 naturally occurring alleles, is the major pathogenicity determinant (3–8); the presence of different *b* alleles is a prerequisite for tumor induction. In addition, the presence of different *b* and different *a* alleles (the *a* locus has 2 naturally occurring alleles) is necessary for maintenance of filamentous growth (8). The *b* locus encodes a polypeptide containing a homeodomain-related motif (9), suggesting that it is a regulatory protein. It is proposed to govern expression of target genes responsible for cell-type specificity (9). The *a* locus may encode a regulatory protein.

In the budding yeast *Saccharomyces cerevisiae*, genes distinct from the mating-type locus (*MAT*) necessary for mating were identified by isolation of mating-defective mutants (10, 11). Many of these genes (the *STE* genes) proved to be expressed in a cell type-specific manner (for example, in **a** or α haploid cells but not in **a**/α diploid cells) and to be targets of the regulatory proteins encoded by *MAT* (refs. 12 and 13; reviewed in ref. 14). Some of these genes code for components of the signaling pathway involved in mating (15, 16). Another target gene is *RME1* (repressor of meiosis), whose expression in haploids prevents initiation of meiosis and sporulation. In the **a**/α diploid, **a**1-α2 represses *RME1* expression, resulting in competence to initiate meiosis and sporulation (17).

I have used a rationale similar to that used in *S. cerevisiae* to identify genes in *U. maydis* distinct from *a* and *b* that may be more directly involved with filament formation, tumor induction, and teliospore production and function. These studies identify three new genes, *fuz1*, *fuz2*, and *rtf1*, that are necessary for filament formation and tumor induction and that may be target genes for the *a* and *b* loci.

MATERIALS AND METHODS

Strains, Media, Growth Conditions, and Biological Methods. *U. maydis* strains are listed in Table 1 (see also Table 5 for diploid strains and their construction) and were grown at 32°C. Media were as described (18). Exponentially growing cells were UV-irradiated as described (19, 20). Conditions for the fuzz reaction are described in the table legends and in ref. 8. Conditions for growth of corn plants (variety B164) and for inoculations are described in the legend to Table 2 (see also Table 4). Germination of teliospores and analysis of meiotic segregants are described in ref. 8.

DNA Isolation and Nucleotide Sequence Determination. Genomic DNA from FBf4-1f, isolated as described (21), was used to construct a library in pUC18. Plasmids containing *b1* were identified as described (9). Nucleotide sequences for both strands were determined by using single- and double-stranded templates with Sequenase (United States Biochemical) and oligonucleotide primers synthesized at the University of California, San Francisco, BioMolecular Research Center.

RESULTS

Isolation of Fuz$^-$ Mutants. An *a1 b1* strain (FB1-49) was mutagenized with UV-irradiation to 8% survival. Colonies were screened for the Fuz$^-$ phenotype after replica mating onto a lawn of an *a2 b2* strain (FB2-47) on charcoal nutrient medium. The wild-type *a1 b1* strain forms white fuzzy colonies under these conditions (the Fuz$^+$ phenotype; see ref. 8) because of formation of dikaryotic filaments. Strains that carry identical *a* or *b* alleles exhibit a Fuz$^-$ phenotype. Thirteen Fuz$^-$ mutant candidates were obtained in 11,000 colonies. Nine gave an altered fuzz reaction upon retesting (Fig. 1): some produced no filaments, others produced very sparse or morphologically altered filaments. Other screens of the same or different strains (*a2 b2*, *a2 b1*, or *a1 b2*) led to the isolation of a total of 80 Fuz$^-$ mutants with fuzz reactions similar to those of the original set of mutants.

Location of *fuz* Mutations Relative to *a* and *b*. Genetic crosses were performed with five of the mutants (strains Fuz1–Fuz5; Table 1) to determine if they carry mutations in *a* or *b* or elsewhere. Each of the five mutants was independently inoculated with a wild-type *a2 b2* strain (FB2). The success of a cross depends on formation of tumors that produce teliospores capable of undergoing meiosis. Tumors containing teliospores were formed in each case. These teliospores exhibited reduced germination efficiency (from <2% to 25%; Table 2) compared

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Table 1. List of strains

Strain	Genotype	Source/ref.
FB1	*a1 b1*	Std. tester strains (see ref. 8)
FB2	*a2 b2*	
FB6a	*a2 b1*	
FB6b	*a1 b2*	
FBD-12	*a1/a2 b1/b2 pan⁻/+ +/ade⁻*	Ref. 8
FBD12-3	*a1/a2 b1/b1*	
FB1-49	*a1 b1 pan⁻*	
FB2-47	*a2 b2 ade⁻*	
FB1-49-2	*a1 b1 pan⁻ met⁻*	UV-derived mutant: of FB1-49
FB6a-91	*a2 b1 ade⁻*	of FB6a
FBf1 (=Fuz1)	*a1 b1 pan⁻ fuz1⁻*	of FB1-49
FBf2 (=Fuz2)	*a1 b1 pan⁻ fuz2⁻*	of FB1-49
FBf3 (=Fuz3)	*a1 b1 pan⁻ rtf1⁻*	of FB1-49
FBf4 (=Fuz4)	*a1 b1 pan⁻ rtf1⁻*	of FB1-49
FBS12-13e	*a2 b1 pan⁻ ade⁻*	Meiotic seg. from FBD-12
FBS12-4c	*a2 b2 pan⁻ ade⁻*	
FBf10-1g,-1i,-5i	*a2 b2 fuz1⁻*	Seg. from cross 1
FBf10-3i,-3c	*a1 b1 fuz1⁻*	
FBf10-1c,-2c,-2e	*a1 b2 fuz1⁻*	
FBf10-5j	*a2 b1 fuz1⁻*	
FBf22-1g	*a1 b1 fuz2⁻*	Seg. from cross 2
FBf22-1a,-1d,-5f	*a1 b2 fuz2⁻*	
FBf22-5c,-5d	*a2 b1 fuz2⁻*	
FBf30-5a,-5i,-6a	*a1 b1 rtf1-3*	Seg. from cross 3
FBf30-1l,-9b,-2d	*a2 b1 rtf1-3*	
FBf4-1c,-1f,-1j	*a1 b1 rtf1-4*	Seg. from cross 4
FBf4-3c,-3e	*a2 b1 rtf1-4*	
FBS37,40	*a2 b2*	Fuz⁺ seg. from cross 5
FBS81,8	*a1 b1*	
FBS13,127	*a2 b1*	
FBS121,124	*a1 b2*	

Crosses were: 1, *a1 b1 fuz1⁻ × a2 b2 fuz1⁺*; 2, *a1 b1 fuz2⁻ × a2 b2 fuz2⁺*; 3, *a1 b1 rtf1-3 × a2 b2 rtf⁺*; 4, *a1 b1 rtf1-4 × a2 b2 rtf⁺*; 5, *a2 b1 fuz2⁻ × a1 b2 fuz1⁻*. Std., standard; Seg., segregant(s).

with >85% germination efficiency for teliospores from wild-type crosses (Table 2). Thus, the mutations in these Fuz⁻ mutants affect teliospore germination.

Meiotic segregants from the above crosses were tested for mating type and mutant phenotype (Table 3). Crosses of the Fuz1⁻ and Fuz2⁻ mutants to a wild-type strain produced Fuz⁺ segregants of all different mating types. Conversely, Fuz⁻ segregants were observed for all mating types. These observations establish that the Fuz1⁻ and Fuz2⁻ strains carry mutations separable from *a* and *b*. Subsequent analysis (described below) shows that these strains carry mutations in different genes. It is not possible to determine rigorously from the data in Table 3 if more than one gene is affected in these mutants. For simplicity, I assume that each strain carries a single mutation and designate the genes so identified as *fuz1* and *fuz2*.

Crosses of the Fuz3⁻ and Fuz4⁻ mutants to a wild-type strain yielded Fuz⁺ segregants, which were primarily of the *b2* mating type (85% and 81%, respectively). Conversely, Fuz⁻ segregants were primarily of the *b1* mating type (97% in each case), indicating tight linkage of the mutation to the *b* locus. In addition, a small number of recombinants were recovered: 6 of 68 segregants from the Fuz3⁻ cross and 6 of 58 segregants from the Fuz4⁻ cross. Recovery of such recombinants indicates that the mutations are separable from the *b* locus. The mutations in Fuz3⁻ and Fuz4⁻ strains are presumed to affect the same gene, which is designated *rtf1* (for *r*egulator of *t*umor *f*ormation).

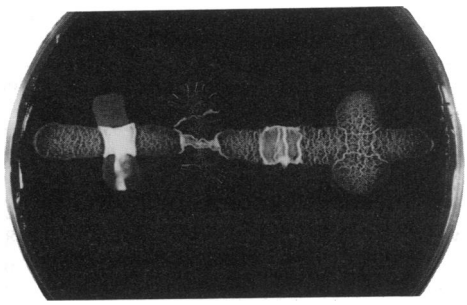

FIG. 1. Fuzz reaction of wild type and *fuz⁻* mutants. Saturated cultures of wild-type *a1 b1* (FB1) and of mutant strains *a1 b1 rtf1–4* (FBf4-f), *a1 b1 fuz1⁻* (FBf10-3i), and *a1 b1 fuz2⁻* (FBf22-5d) (from left to right, respectively) were cross-streaked against wild-type *a2 b2* (FB2) (horizontal line) on charcoal nutrient medium and incubated for 48 hr at room temperature.

Cell and Growth Phenotypes of *fuz1* and *fuz2*. Both the *fuz1⁻* and *fuz2⁻* mutations lead to altered cell morphology: mutant cells are approximately twice as long and half as wide

Table 2. Phenotype of Fuz⁻ mutants

Cross	Fuz phenotype	Tumor induction	Teliospore production (%)
1. *fuz⁺ × fuz⁺*	+	+	+ (85)
2. *fuz1⁻ × fuz1⁺*	+/−	+	+ (16)
3. *fuz1⁻ × fuz1⁻*	+/=	+/=	− (NA)
4. *fuz2⁻ × fuz2⁺*	+/−	+	+ (17)
5. *fuz2⁻ × fuz2⁻*	−	+	+ (<0.5)
6. *fuz1⁻ × fuz2⁻*	−	+/−	+ (32)
7. *rtf1–4 × rtf1⁺*	+/=	+	+ (2)
8. *rtf1–3 × rtf1⁺*	+/=	+	+ (24)

Fuz phenotype was determined by cross-streaking the strains indicated below on charcoal nutrient medium (8). The reaction was scored at 16, 24, 48, and 72 hr of incubation at room temperature. All nine Fuz⁻ segregants from the cross *a1 b1 fuz1⁻ × a2 b2 fuz1⁺* listed in Table 1 were tested with the appropriate tester (cross 2) or among themselves (cross 3). All six Fuz⁻ segregants from the cross *a1 b1 fuz2⁻ × a2 b2 fuz2⁺* listed in Table 1 were tested with the appropriate tester (cross 4) or among themselves (cross 5). Fuz⁻ strains for cross 6 were FBf10-5j (*a2 b1*) × FBf22-1a,-1d,-5f (*a1 b2*); FBf10-1c,-2c (*a1 b2*) × FBf22-5c,-5d (*a2 b1*). All five Fuz⁻ segregants of the cross *a1 b1 rtf1–4 × a2 b2 rtf1⁺* and all five Fuz⁻ segregants of the cross *a1 b1 rtf1–3 × a2 b2 rtf1⁺* listed in Table 1 were tested with the appropriate tester strain (crosses 7 and 8, respectively). For cross 1, Fuz⁺ strains were the standard testers FB1 × FB2 and FB6a × FB6b (Table 1). Tumor formation was assayed after coinoculation of 5-day-old corn seedlings, variety B164, with 0.2 ml of a 1:1 mixture of saturated cultures (2×10^8 cells per ml) of the strains indicated below. Tumor formation was detected as early as 3 days after inoculation, and its development was followed for 1 month. Cross 3 involved 24 independent inoculations with FBf10-3i (*a1 b1*) × FBf10-1g,-1i,-5i (*a2 b2*); FBf10-2c (*a1 b2*) × FBf10-5j (*a2 b1*). Cross 5 involved 8 independent inoculations with FBf22-5d (*a2 b1*) × FBf22-5a,-5f (*a1 b2*). Cross 6 involved 28 independent inoculations with FBf22-1g (*a1 b1*) × FBf10-1g,-1i,-5i (*a2 b2*); FBf22-5c (*a2 b1*) × FB10-2c (*a1 b2*); and FBf22-1a,-5f (*a1 b2*) × FBf10-5j (*a2 b1*). For crosses 2, 4, 7, and 8, the above mutant strains were coinoculated with the appropriate tester strains at least twice. *fuz⁺ × fuz⁺* crosses were FB1 × FB2 and FB6a × FB6b. Teliospore production was assessed by direct visual inspection of the tumors (teliospores appear as dark brown specks in the whitish green tumors 8–10 days after inoculation) and by microscopic observation of tumor slices. Teliospore germination was examined microscopically on nutrient agar slabs after removal from tumors. Numbers in parentheses indicate % teliospore germination. NA, not applicable; Fuz⁺, full filament formation; Fuz⁺/⁻, reduced or altered filament formation; Fuz⁺/⁼, very reduced filament formation; Fuz⁻, no filament formation. Tum⁺, full tumor induction; Tum⁺/⁻, reduced tumor size or delayed reaction; Tum⁺/⁼, very small tumors and altered distribution.

Table 3. Segregation of *fuz*$^-$ mutations with respect to the *a* and *b* loci

Cross	Segregants		Mating type of segregant, no.			
	Fuz phenotype	No.	*a1 b1*	*a2 b2*	*a1 b2*	*a2 b1*
Fuz1$^-$ × wt	+	26	6	8	1	11
	−	21	3	5	12	1
Fuz2$^-$ × wt	+	34	4	20	8	2
	−	14	2	3	6	3
Fuz3$^-$ × wt	+	33	1	16	12	4
	−	35	18	0	1	16
Fuz4$^-$ × wt	+	26	4	11	10	1
	−	32	15	0	1	16

The Fuz$^-$ strains were the original mutants (strains Fuz1–Fuz4), which were derived from an *a1 b1* strain (FB1-49) as described in the text. They were crossed to a wild-type (wt) *a2 b2* strain (FB2), and the mating type of meiotic segregants was deduced by reaction with standard testers (8) (Table 1). The Fuz5 mutant gave a complex segregation pattern (data not shown) and was not characterized further. The greater number of Fuz$^+$ than Fuz$^-$ segregants recovered in the Fuz2$^-$ cross has several possible explanations—reduced penetrance of the mutation, presence of unlinked genetic modifiers, requirement of two different mutations for the phenotype, or reduced viability of the Fuz$^-$ progeny. Plant inoculations, teliospore germination, and mating reactions were performed as described in the legend to Table 2 and in ref. 8. The total number of segregants analyzed in each cross derives from at least six different teliospores, and the reaction for each segregant was analyzed on two different occasions. Fuz phenotype: +, wild-type reaction; −, mutant reaction.

as the parental strain. This phenotype cosegregates with the Fuz phenotype. Growth rates of the mutants approximate those of the wild-type parental strain.

Tumor Induction. To determine the effect of the *fuz1*$^-$ and *fuz2*$^-$ mutations on tumor induction and teliospore function, plants were coinoculated with sets of *fuz*$^-$ haploids. The tumors produced by *fuz1*$^-$ mutants are very small compared with those from control crosses (Table 2, lines 1–3) and are localized mainly along the leaf midrib rather than on the entire leaf as observed in control inoculations. No teliospores were produced. Thus, the *fuz1* gene appears to be necessary for filament formation, production of full-sized tumors, and formation of teliospores.

Coinoculation of plants with *fuz2*$^-$ haploids leads to tumor induction similar to that of the controls (Table 2, lines 1, 4, and 5), though tumor formation is delayed 1–2 days compared with the normal situation. *fuz2*$^-$ × *fuz2*$^-$ tumors produced teliospores, but these teliospores were incapable of germination (0 of 200 germinated; Table 2). Thus, the *fuz2* gene appears to be necessary for filament formation and for germination of teliospores.

The presence of a *fuz*$^-$ mutation in both mating partners causes a more severe defect on filament formation (Table 2, lines 3, 5, and 6) than when only one of the mating partners is mutant (Table 2, lines 2 and 4).

Allelism Tests of *fuz1* and *fuz2*. The phenotypes exhibited by *fuz1*$^-$ or *fuz2*$^-$ mutants suggest that the mutations are in different genes. Crosses were performed between *fuz1*$^-$ and *fuz2*$^-$ strains to test whether *fuz1* and *fuz2* are allelic. Of 120 meiotic segregants from 21 different teliospores from one cross, 30 were Fuz$^+$, 66 were Fuz$^-$, and 24 gave other reactions (see below). The large fraction of Fuz$^+$ segregants recovered indicates that the Fuz1$^-$ and Fuz2$^-$ strains carry mutations in different genes. Backcrosses of the Fuz$^+$ segregants (Table 1) to a wild-type strain confirmed that they are genetically *fuz*$^+$ (data not shown). The 24 unusual segregants (dual maters and fuzzy constitutives) may be *fuz1*$^-$ *fuz2*$^-$ double mutants or may have arisen from meiotic nondisjunction and have not been studied further.

Tumor Induction by *rtf1*$^-$ Mutants. Fuz3$^-$ and Fuz4$^-$ strains (Table 1) exhibited an unexpected behavior with respect to tumor formation. Coinoculation of plants with haploids carrying *identical b* alleles and an *rtf1* mutation yielded tumors identical in size and distribution (Table 4, experiment A) to those produced by two haploids carrying different *b* alleles and a wild-type *rtf1* gene (Table 4, experiment D). These observations indicate that the *rtf1*$^-$ mutation bypasses the requirement for the presence of different *b* alleles. If the *rtf1*$^-$ mutations bypass the need for combinations of different *b* alleles, then a haploid *rtf1*$^-$ strain (which has only one type of *b* allele) might be pathogenic. Indeed, one of the mutants, *rtf1–4*, appears to be weakly pathogenic in the haploid state: 20% of inoculations with pure cultures yielded tumors (Table 4, experiment C). *rtf1*$^-$ haploids are not as pathogenic in single infection as in coinfections. This difference may indicate that mating itself or the presence of different *a* alleles is necessary for establishment of a fully pathogenic form.

***rtf1*$^-$ Mutations Are Recessive.** To determine dominance or recessiveness of the *rtf1*$^-$ mutations, a set of diploids between *rtf1*$^-$ and *rtf1*$^+$ strains was constructed. Inoculation of plants with *a1/a2 b1/b1 rtf1-4/rtf1*$^+$ or with *a1/a2 b1/b1 rtf1-3/rtf1*$^+$ diploids did not induce tumor formation (Table 5, experiment A). Thus, *rtf1–4* and *rtf1–3* are both recessive to *rtf1*$^+$. *a1/a2 b1/b2 rtf*$^-$*/rtf1*$^+$ diploids resulted in tumor induction in all inoculations (Table 5, experiment B). The recessiveness of *rtf1*$^-$ indicates that these mutations result in the loss or reduction of Rtf function.

***rtf1* Is Distinct from *b*.** The crosses described above indicate that the *rtf1*$^-$ mutation is close to but separable from the *b*

Table 4. Tumor production after coinoculation with *rtf1*$^-$ strains

Exp.	Strains	Tum$^+$	Tum$^-$	% tumor production
A	*a1 b1 rtf1–4* + *a2 b1 rtf1–4*	25	7	78*
	a1 b1 rtf1–3 + *a2 b1 rtf1–3*	12	13	48*
B	*a1 b1 rtf1–4* + *a2 b2 rtf1*$^+$	9	0	100†
	a1 b1 rtf1–3 + *a2 b2 rtf1*$^+$	5	0	100†
C	*ax b1 rtf1–4* alone	3	12	20‡
	ax b1 rtf1–3 alone	0	9	<10
D	*a1 b1 rtf1*$^+$ + *a2 b2 rtf1*$^+$	10	0	100
E	*a1 b1 rtf1*$^+$ + *a2 b1 rtf1*$^+$	0	10	<10

Conditions for growth of strains, plant inoculations, assessment of teliospore formation, and abbreviations are as described in the legend to Table 2. Plants were maintained in a Conviron chamber (14 hr of light at 28°C; 10 hr of dark at 20°C). In experiment A, coinoculations with *rtf1*$^-$ strains (Table 1) were FBf4-1f + FBf4-3c (9) or + FBf4-3e (8); and FBf4-1j + FBf4-3c (7) or + FBf4-3e (4). For *rtf1–3* strains, coinoculations were FBf30-6a + FBf30-9b (7); FBf30-5i + FBf30-2d (8); and FBf30-5i + FBf30-9b (5) or + FBf30-1l (5). In experiment B, coinoculations with *rtf1–4* strains were FBf4-1f + FB2 (5); and FBf4-3c + FB6b (4). For *rtf1–3* strains, coinoculations were FBf30-6a + FB2 (3); and FBf30-9b + FB6b (2). In experiment C, the *rtf1–4* strains were FBf4-1f (4), -1j (4), -3c (4), and -3e (3); the *rtf1–3* strains were FBf30-6a (3), -5i (3), and -9b (3). Numbers in parentheses are numbers of independent inoculations. *ax* = *a1* or *a2*. In experiments D and E, the strains were FB1 + FB2 and FB1 + FB6a, respectively.

*The tumors were indistinguishable from those obtained by coinoculation of wild-type strains carrying different *b* alleles (experiment D) with respect to size, distribution, time course of development, teliospore production, and induction of anthocyanin pigmentation. The fraction of inoculated plants producing tumors was less than in the controls.

†Tumor development and distribution were similar to that observed by coinoculation of wild-type strains (experiment D), but the tumors were smaller than in control inoculations.

‡Only a few very small tumors were observed, and their development was delayed compared with tumors from control inoculations (experiment D). Induction of anthocyanin pigmentation was weak.

Table 5. Tumor production by *rtf1*$^-$/*rtf1*$^+$ diploids

Exp.	Strain	Tum$^+$	Tum$^-$	% tumor production
A	*a1/a2 b1/b1 rtf1*$^-$/*rtf1*$^+$	0	39	<3
B	*a1/a2 b1/b2 rtf1*$^-$/*rtf1*$^+$	18	0	100
C	*a1/a2 b1/b1 rtf1*$^+$/*rtf1*$^+$	0	10	<10
D	*a1/a2 b1/b2 rtf1*$^+$/*rtf1*$^+$	6	0	100

In experiment A, the *rtf1–4*/*rtf1*$^+$ diploids are FBD91-f (6), −j (6), FBD13-f (6), and -j (6). The *rtf1–3*/*rtf1*$^+$ diploids are FBD91-305 (6), -306 (2), FBD2-309 (5), and FBD13-306 (2). In experiment B, the *rtf1–4*/*rtf1*$^+$ strains are FBD4-f (3) and -j (6). The *rtf1–3*/*rtf1*$^+$ diploids are FBD4-305 (6) and -306 (3). In experiments C and D, the diploid strains are FBD12-3 and FBD-12, respectively. Numbers in parentheses are numbers of independent inoculations. FBD91-f, -j, -305, and -306 are diploids (*a1/a2 b1/b1 rtf1*$^-$/*rtf1*$^+$ *ade*$^-$/+ *hyg*^{s}/*hyg*^{r}) obtained by mating FB91H with FBf4-1f, FBf4-1j, FBf30-6a, and FBf30-5a, respectively, with selection for hygromycin-resistant (*hyg*^{r}) prototrophs on minimal medium containing 200 μg of hygromycin per ml. FBD13-f, -j, and -306 are diploids (*a1/a2 b1/b1 rtf1*$^-$/*rtf1*$^+$ *ade*$^-$/+ *pan*$^-$/+ *hyg*^{s}/*hyg*^{r}) obtained by mating FB13eH with FBf4-1f, FBf4-1j, and FBf30-6a, respectively, with selection as for FBD91-f. FBD2-309 is a diploid (*a1/a2 b1/b1 rtf1*$^-$/*rtf1*$^+$ +/*met*$^-$ +/*pan*$^-$) obtained by mating FB2H with FBf30-9b. FBD4-f, -j, -305, and -306 are diploids (*a1/a2 b1/b2 rtf1*$^-$/*rtf1*$^+$ *ade*$^-$/+ *pan*$^-$/+ *hyg*^{s}/*hyg*^{r}) obtained by mating FB4cH with FBf4-1f, FBf4-1j, FB30-5a, and FB30-6a, respectively, with selection as for FBD91-f. Plant inoculations, other conditions for strain manipulations, and abbreviations are described in the legend to Table 2. FB6a-91 and FB1-49-2 (Table 1) are auxotrophic derivatives of FB6a and FB1-49, respectively, and were obtained as described in ref. 8. FB-D12 and FB-D12-3 are diploids described in ref. 8 (see Table 1). FBS12-13e and FBS12-4c are haploid meiotic segregants (Table 1) from FB-D12. FB2H, FB91H, FB13eH, and FB4cH are *hyg*^{r} derivatives of FB1-49-2, FB6a-91, FBS12-13e, and FBS12-4c, respectively, and were obtained by transformation with plasmid pcM54 (22) by the procedure of Tsukuda *et al.* (22).

locus. The possibility exists that the segregants scored as recombinants were instead due to unlinked genetic modifiers or to reduced penetrance of the mutation. If so, the *rtf1* mutations might affect the *b* locus itself. This was directly examined by cloning the *b1* allele of the *rtf1–4* strain (FBf4-1f) and determining the nucleotide sequence of the *b1* open reading frame. No changes were detected in the open reading frame or in a 412-base-pair region upstream of the starting ATG. The 3′ untranslated region has not been sequenced. Although the mutation could reside in this region, these analyses together with the recovery of putative recombinants indicate that the *rtf1*$^-$ mutation is in a gene near but distinct from *b*.

DISCUSSION

Three genes, *fuz1*, *fuz2*, and *rtf1*, necessary for completion of steps in the life cycle of *U. maydis* have been identified. These genes were identified by isolation of mutants of an *a1 b1* strain unable to form filaments when mated with an *a2 b2* strain. *fuz1* and *fuz2* are not linked to *a* and *b*, whereas *rtf1* appears to be closely linked to *b*. Because the two master regulatory loci, *a* and *b*, are necessary for maintenance of filamentous growth, these new genes may represent targets for *a* or *b*.

Possible Roles of *fuz1* and *fuz2*. The *fuz1* gene is necessary for filament formation on nutrient medium, for normal tumor induction, and for teliospore formation. *fuz1*$^-$ mutants produce only small tumors that are few in number and restricted to certain leaf areas. These tumors are devoid of teliospores. The defect in tumor formation of *fuz1*$^-$ mutants could be due to inefficient mating, to poor growth of the filamentous form within the plant, or to inadequate signaling between *U. maydis* and its host. Failure of *fuz1*$^-$ mutants to produce teliospores may be due to a defect in the differentiation of hyphae within the tumors (1, 2).

The *fuz2* gene is necessary for filament formation on nutrient medium and for teliospore germination. *fuz2*$^-$ mutants are able to form tumors and teliospores, but these teliospores are incapable of germination. During teliospore germination, the thick cell wall of the teliospore breaks down and a short filament (the promycelium) is formed into which the diploid nucleus migrates for the ensuing meiotic divisions (23, 24). *fuz2* may encode an enzyme necessary for cell-wall breakdown during germination, or its product may be necessary for growth of the promycelium.

The altered cell shape of *fuz1* and *fuz2* mutants leads to the suggestion that these genes may code for components of the cytoskeleton. A deficiency in a cytoskeletal component might interfere with localization of materials at the growing tip of the bud and the filament or with localization of material necessary for teliospore maturation or germination. In *Schizophyllum commune*, a wood-rotting Basidiomycete, genes distinct from the mating-type factors that are necessary for nuclear migration (25) could also be imagined to encode cytoskeletal components (26, 27) and to be the targets of the *B* mating factor, which governs nuclear migration (28).

Possible Roles for *rtf1*. The results presented here have shown that recessive mutations in *rtf1* bypass the need for different *b* alleles: two *rtf1*$^-$ haploids carrying the same *b* allele induce tumors that are identical to those obtained by coinoculation of strains carrying different *b* alleles. A variety of hypotheses (Fig. 2) can explain this observation. In hypothesis I, *rtf1* is a negative regulator of initiation of tumor

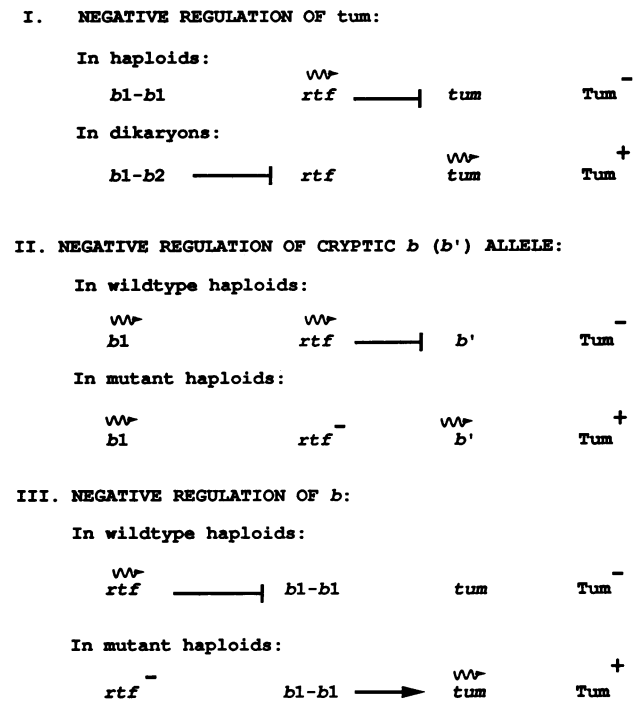

FIG. 2. Possible roles for *rtf1*. Hypotheses to explain the observation that recessive mutations in the *rtf1* gene bypass the need for different *b* alleles for tumor induction. In hypothesis I, *rtf1* codes for a negative regulator of tumor induction, which is expressed in haploids and inhibits tumor formation. In dikaryons, *b1* and *b2* form a heteromultimer that represses *rtf1* and thus leads to tumor formation. In hypothesis II, *rtf1* codes for a negative regulator of a cryptic *b* allele (*b'*). In *rtf1*$^-$ mutant haploids, the *b'* product interacts with *b1* to create a heteromultimer that leads to tumor induction. In hypothesis III, *rtf1* is a negative regulator of *b*, which is essential for tumor induction. In *rtf1*$^-$ mutant haploids, *b* stimulates expression of *tum* genes. In wild-type dikaryons, either the *b1-b2* heteromultimer is insensitive to *rtf1*, or *b1-b2* inhibits *rtf1*.

induction. In a *b1/b2* dikaryon, the regulatory protein formed by interaction of different *b* monomers represses the *rtf1* gene, thus allowing tumor induction by this cell type. In haploid cells, *rtf1* is not repressed; consequently, its product inhibits tumor formation (Fig. 2). This situation is analogous in some respects to control of sporulation in *S. cerevisiae*, in which haploid cells express *RME1*, an inhibitor of initiation of meiosis and sporulation. In **a**/α diploids, the **a**1-α2 combinatorial activity represses *RME1*, thereby allowing initiation of meiosis and sporulation (17). Recessive mutations in *RME1* bypass the requirement for **a**1-α2 (29). Thus, *rtf1* and *RME1* appear to be analogues in that they are both negative regulators of sexual development of the cell type resulting from mating; and both may be subject to negative regulation (by *b1-b2* or **a**1-α2 multimer, respectively).

In a second hypothesis, *rtf1* is a negative regulator of a cryptic *b* allele (designated *b'*) (Fig. 2), or *rtf1* itself is the cryptic *b* allele. The recessive *rtf1* mutation allows expression of *b'*, which has a different allele specificity than *b1* (the allele present in the mutant). Expression of the *b'* allele thus results in the presence of different *b* alleles in the cell and consequently in ability to initiate tumor induction. In *S. cerevisiae*, recessive mutations in any of the four *SIR* genes allow expression of silent copies of *MAT*, resulting in ability to initiate meiosis and sporulation (30). There is presently no evidence for cryptic *b* alleles in *U. maydis*, but there is precedent for functionally redundant mating type loci in other Basidiomycetes (28).

In a third hypothesis, *rtf1* is a negative regulator of *b*. In wild-type haploids, *rtf1* inhibits expression or activity of *b*, which is essential for tumor induction; consequently, tumors are not formed. *rtf1*$^-$ mutant haploids have *b* activity and thus activate the *tum* genes. Another postulate of this hypothesis is that a *b1–b2* heteromultimer formed in a dikaryon may either be insensitive to *rtf1* or may inhibit *rtf1*.

In addition to its role in tumor production, *rtf1* is also necessary for filament formation. One possibility is that *rtf1* is a positive regulator of a gene (*fil*) necessary for establishment of filamentous growth and the *fil* product, like the products of *fuz1* and *fuz2*, needs to be present in both partners at the time of mating.

Although the precise roles of *fuz1*, *fuz2*, and *rtf1* cannot be deduced at present, it seems likely that they shall be of interest from the standpoint of both gene regulation and cell biology. Cloning of the *U. maydis fuz* genes will make it possible to determine whether their expression is indeed regulated by *a* or *b*. The cloned *fuz* genes will also provide a probe for examining other phytopathogenic fungi for *fuz* homologues, which might be determinants of pathogenicity.

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