Different \(a\) alleles of \textit{Ustilago maydis} are necessary for maintenance of filamentous growth but not for meiosis

(corn smut fungus/plant pathology/tumor induction/mating)

\textbf{Flora Banuett and Ira Herskowitz}

Department of Biochemistry and Biophysics, School of Medicine, University of California, San Francisco, San Francisco, CA 94143

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\textbf{ABSTRACT} Two loci (the incompatibility or mating type loci), \(a\) and \(b\), govern the life cycle of \textit{Ustilago maydis}, a fungal pathogen of corn. \textit{U. maydis} diploids heterozygous at both \(a\) and \(b\) \((a\# b\#)\) form mycelial colonies (Fuz\(^*\) phenotype), induce tumors, and produce teliospores able to undergo meiosis. We report here the isolation and characterization of nonmycelial (Fuz\(^-\)) derivatives. These Fuz\(^-\) derivatives have allowed us to examine the requirement of \(a\#\) and \(b\#\) in maintenance of filamentous growth and tumor-inducing ability. The Fuz\(^-\) diploids are of four classes: two are inferred to be homozygous for \(b (a\# b\#)\); the other two are shown to be homozygous for \(a (a = b\#)\). These observations confirm the requirement for \(b\#\) and reveal the requirement for \(a\#\) in filamentous growth. \textit{U. maydis} is thus like other basidiomycetes that have two loci governing growth of the dikaryotic filament. The mating behavior of the Fuz\(^-\) diploids indicates that heterozygosity at \(a\) or \(b\) does not block mating. Although both \(a = b\#\) and \(a\# b = b\#\) are nonmycelial, they differ in that \(a\# b\) diploids are nonpathogenic, whereas \(a = b\#\) diploids are pathogenic and produce teliospores able to undergo meiosis. These findings substantiate previous, more limited observations. They demonstrate that ability to induce tumors and produce normal teliospores requires different alleles at \(b\) but not at \(a\).

\textit{Ustilago maydis}, a basidiomycetous fungus, causes corn smut disease, which is characterized by tumors (galls) on leaves, stems, tassels, and ears. The fungus exhibits two distinct forms in its life cycle (1, 2): a unicellular nonpathogenic haploid form and a filamentous pathogenic dikaryotic form. Cell fusion between haploid forms gives rise to the dikaryotic filament, which can grow only in the plant, where it induces tumor formation. Differentiation of hyphal cells within the tumors results in nuclear fusion and formation of diploid teliospores (3, 4). Teliospores are unable to grow vegetatively but germinate and undergo meiosis to produce haploid unicellular segregants. Production of the two forms and completion of the life cycle are governed by two mating type or incompatibility loci, \(a\) and \(b\) (2). Two naturally occurring alleles have been identified for the \(a\) locus (5) and at least 25 have been identified for the \(b\) locus (5–7). Only matings between haploids that differ at both loci result in filament formation and tumor production (8). For example, matings between \(a1 b1\) and \(a2 b2\) haploids yield tumors, whereas those between \(a1 b1\) and \(a2 b1\) do not.

Formation of dikaryotic hyphae is a two-step process: first, haploid cells fuse to form a dikaryotic cell; second, the dikaryon grows as a filament. We refer to the first step as establishment of dikaryosis and the second step as maintenance of dikaryosis and the filamentous state. The current view of the requirements for different alleles of the incompatibility loci of \textit{U. maydis} in cell fusion and filamentous growth comes mainly from studies in which haploids of different genotypes are mixed on nutrient media and formation of dikaryotic filaments is assessed (7, 8). It is not possible to determine whether \(a\#\) or \(b\#\) or both are required for growth of the dikaryotic filament from this type of experiment because failure to observe filamentation may reflect either failure of cells to fuse or failure of the dikaryon to grow.

Diploid cells make it possible to study postfusion events—in particular, maintenance of the filamentous state and tumor-inducing ability. Diploids capable of vegetative growth are not a normal state in the life cycle of \textit{U. maydis}, but they can be constructed in the laboratory (see Results) or they can occur in nature when teliospores fail to undergo meiosis (ref. 9; F.B., unpublished data). A limited study of diploids indicates the importance of heterozygosity at \(b\) in filamentous growth: a diploid strain differing at both \(a\) and \(b\) \((a\# b\#)\) forms filaments, whereas an \(a\# b = b\#\) diploid does not (6, 10). In addition, \(a\# b = \) strains are not pathogenic, showing that heterozygosity at \(b\) is also necessary for tumor formation (5, 9, 10).

In this paper, we present analysis of a set of isogenic diploid strains to address the need for \(a\#\) and \(b\#\) in maintenance of the filamentous state and tumor-inducing ability, independently of any role that \(a\) and \(b\) may have on cell fusion. Some of our conclusions are new: in particular, we demonstrate that different alleles at the \(a\) locus are necessary, together with \(b\), for maintenance of filamentous growth. We also show that heterozygosity at \(a\) is not necessary for teliospore germination or for meiosis. In addition, we show that heterozygosity at \(a\) or \(b\) does not block mating. Other conclusions support earlier contentions and provide additional documentation: specifically, we show that heterozygosity at \(a\) is not a requirement for pathogenicity of a diploid.

\textbf{MATERIALS AND METHODS}

\textbf{Strains, Media, Growth Conditions, UV Irradiation.} \textit{U. maydis} strains are listed in Table 1. Media are as described (11). Strains were grown at 32°C. Exponentially growing cells were irradiated as described (11-13).

\textbf{Fuz Reaction on Charcoal Media.} Saturated cultures of strains to be tested were co-spotted or cross-streaked on charcoal nutrient medium (11). Plates were sealed with Parafilm and incubated at room temperature to facilitate scoring the Fuz phenotype. Formation of hyphae was assessed after overnight incubation and was followed for 2–3 days using a Zeiss stereo microscope.

\textbf{Construction of Diploids.} Diploids were obtained as indicated (11). Compatible haploids carrying complementing auxotrophic markers were co-spotted and incubated on nutrient medium at room temperature for 24 hr. The cell mixture was transferred to minimal medium, where diploid cells but not dikaryons are able to grow. After several days, vigorously growing patches appeared, from which individual diploid colonies were isolated.
Plant Growth and Infections. Corn plants (variety Golden Bantam or B164) were grown under controlled environmental conditions (14-hr light/10-hr dark cycle, 28°C) in a Conviron growth chamber. One-week-old plants were inoculated as described (5). Tumors appeared 5–8 days after inoculation, and teliospores were harvested ~2 weeks postinoculation.

Teliospore Germination and Spore Analysis. Teliospores were harvested from tumors as described (11) and spread on slabs of nutrient agar with the aid of a micromanipulator. They were germinated at 32°C; micromycelium appeared in 2–3 days. Meiotic segregants were isolated from these microcolonies by streaking on nutrient medium.

RESULTS

Construction of Diploids Heterozygous at a and b. Mating between two U. maydis haploids differing at a and b ordinarily produces a dikaryon. Diploid strains FB-D11 and FB-D12 were obtained by selecting for prototrophs in matings of an a1 b1 pan⁻ haploid with an a2 b2 ade⁺ haploid (ref. 11; see Materials and Methods). These strains form mycelial colonies on charcoal medium (the Fuz⁻ phenotype). In contrast, the haploid parents form compact colonies devoid of filaments (the Fuz⁺ phenotype). FB-D11 and FB-D12 and also solopathogenic—that is, a pure culture induces tumors when inoculated singly into a plant. These properties exhibited by a ≠ b⁻ diploids have been observed previously (5, 7, 9, 10).

The genotype of these strains was confirmed by analysis of the segregants obtained upon meiosis of teliospores. Mature teliospores (diploid spores) were recovered from tumors in corn plants that had been independently inoculated with pure cultures of FB-D11 and FB-D12. (Teliospore formation is absolutely dependent on growth within the plant.) Teliospores undergo meiosis upon germination on nutrient agar (3, 4), and a single teliospore gives rise to a microcolonial containing a mixture of all meiotic segregants. Individual colonies representing single meiotic segregants were recovered after streaking the microcolonies. Sixty progeny from 13 teliospores of FB-D12 were analyzed for mating type and auxotrophic markers. Mating type was determined on charcoal plates (2) by cross-streaking with four testers (a1 b1, a2 b2, a2 b1, a1 b2); a given haploid mates with only one of the four tester strains (carrying different a and b alleles) as shown in Table 1 and Fig. 1. The results for FB-D12 are presented in Table 3. Similar results were obtained for FB-D11 (data not shown). Four different mating types were recovered among the progeny in approximately equal numbers as expected for a strain heterozygous at two independently segregating loci. The data also demonstrate that FB-D12 is heterozygous for pan and ade. (The underrepresentation of adenine-requiring segregants may be due to poor growth of these strains.) These data demonstrate that FB-D12 and FB-D11 are of genotype a1/a2 b1/b2 pan⁺/- ade⁻/-.

Behavior of Fuz⁻ Diploid Strains. a≠ b⁻ diploids are, as indicated above, Fuz⁺ on charcoal medium. Fuz⁻ derivatives were obtained after irradiation of both FB-D12 and FB-D11 with low doses of UV irradiation (15–30% survival). The Fuz⁻ strains obtained were analyzed for mating reaction (fuzz reaction) by cross-streaking 9 isolates from FB-D11 and 19 from FB-D12 against the four testers (a1 b1, a2 b2, a2 b1, a1 b2). The Fuz⁻ derivatives have the distinctive characteristic of mating with two different haploid testers, in contrast to haploid strains, which mate only with one tester strain (Fig. 1; Table 2).

The Fuz⁻ strains are of four types (Fig. 1; Table 2): Class I gives a positive reaction with testers having a b2 allele, and class II gives a positive reaction with testers having a b₁ allele, independent of the a allele carried by the testers. We infer class I to be of genotype a1/a2 b1/b1 and class II to be a1/a2 b2/b2 (Table 2). Class III derivatives give a positive fuzz reaction with testers having an a2 allele, and class IV gives a positive reaction with testers having an a₁ allele, regardless of the b allele carried by the tester strains. The simplest explanation for the latter two classes is that they have become homozygous at a: Class III and class IV are thus inferred to be a1/a1 b1/b2 and a2/a2 b1/b2, respectively. Upon prolonged incubation on charcoal medium, class III and IV strains, in isolation, exhibit incipient hyphae that are

Table 2. Mating reaction of haploid strains and Fuz⁻ derivatives of a1/a2 b1/b2 diploids

<table>
<thead>
<tr>
<th>Strains</th>
<th>a1 b1</th>
<th>a2 b2</th>
<th>a1 b2</th>
<th>a2 b1</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>Tester strains</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>FB2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>a2 b2</td>
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<tr>
<td>FB1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>a1 b1</td>
</tr>
<tr>
<td>FB6a</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>a2 b1</td>
</tr>
<tr>
<td>FB6b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>a1 b2</td>
</tr>
<tr>
<td>Class I</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>a1/a2 b1/b1</td>
</tr>
<tr>
<td>Class II</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>a1/a2 b2/b2</td>
</tr>
<tr>
<td>Class III</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>a1/a1 b1/b2</td>
</tr>
<tr>
<td>Class IV</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>a2/a2 b1/b2</td>
</tr>
</tbody>
</table>

+, formation of hyphae; −, no hyphae. Genotypes for classes I and II are inferences because no progeny are produced. Genotypes for classes III and IV were confirmed by progeny test. The number of Fuz⁻ derivatives of classes I, II, III, and IV obtained were 1, 2, 6, 0, respectively, for FB-D11, and 7, 0, 6, 6, respectively, for FB-D12.
Fig. 1. Mating reaction of haploid and Fuz⁻ diploid strains. Saturated cultures of haploid strains (top four horizontal lines) and Fuz⁻ diploids (bottom four horizontal lines) were cross-streaked against haploid testers al b1, a2 b2, a1 b2, and a2 b1 (from left to right) on charcoal medium and incubated overnight at room temperature. Strains in horizontal lines are (from top to bottom) a2 b2, a1 b1, a2 b1, a1 b2, a1/a2 b1/b1 (class I), a1/a2 b2/b2 (class II), a1/a1 b1/b2 (class III), and a2/a2 b1/b2 (class IV). The arrangement of strains in this figure is the same as in Table 2. Surface ridges are evident in the a= b+ diploids.

We presume that the Fuz⁻ derivatives are mitotic recombinants homozygous for a or b, but we cannot exclude the possibility that they are hemizygous—for example, a1/a2 b1/− instead of a1/a2 b1/b1. It is likely that the strains are homozygous because U. maydis diploids are very stable and chromosome loss is not readily observed (R. Holliday, personal communication). Furthermore, low doses of UV irradiation are known to stimulate mitotic recombination in U. maydis (9, 12).

Two other possible genotypes that would result in a Fuz⁻ phenotype are readily excluded. (i) Homozygosity at both a and b (a= b+); such strains would mate with only one of the four testers. (ii) Homozygosity for another b allele—for example, b3; in this case, the strains would mate with all of the testers. Neither of these mating reactions was observed in the analysis of many independently obtained Fuz⁻ diploids.

The results confirm that loss of heterozygosity at b results in inability to form hyphae. They also clearly show that failure to form filaments can also be due to loss of heterozygosity at a. Thus, the a locus, together with b, is necessary for maintenance of filamentous growth of U. maydis diploids.

The fuzz reactions also demonstrate that the presence of different alleles at a or b does not block the mating reaction. a1/a1 b+ and a2/a2 b+ diploids are compatible (give Fuz⁺ reaction) with a2 or a1 testers, respectively, which demonstrates that heterozygosity at b does not inhibit mating. Similarly, diploids inferred to be a b1/b1 and a b2/b2 are compatible with b2 or b1 testers, respectively, demonstrating that heterozygosity at a alone does not inhibit mating. These conclusions are further supported by the observation that Fuz⁻ diploids are incompatible (no fuzz reaction) with members of their own class but compatible with those of the other classes (data not shown). In all these reactions, the strength and the time course of appearance of mycelial growth was indistinguishable from that seen in matings between haploid strains.

**Pathogenicity of Fuz⁻ Diploid Strains.** We tested the pathogenicity of the four different types of Fuz⁻ diploids by independent inoculation of corn plants with each of the different diploids and by assessment of tumor formation. As controls, we inoculated with the Fuz⁺ diploids and also with compatible haploid strains. The results are summarized in Table 4. The Fuz⁺ (a b+) parental diploids (FB-D11 and FB-D12) induce tumors (i.e., they are solopathogenic), as had been shown previously (5, 9, 10). The diploids homozygous for b were nonpathogenic, confirming results obtained by others (5, 10). In contrast, diploids homozygous for a were pathogenic and produced teliospores. No differences were observed between a1/a1 and a2/a2 homozygotes or between a= b+ and a b+ strains with respect to virulence (tumor size, distribution) or teliospore production, whether the inoculations were done at high or low densities. We conclude that although different alleles at both a and b are a prerequisite for maintenance of filamentous growth, only different alleles at b are necessary for maintenance of tumor-inducing ability and teliospore formation by diploid strains.

**Analysis of Meiotic Segregants from a= b+ Diploids.** Fuz⁻ diploids of presumed a= b+ genotype produce teliospores capable of germinating and undergoing meiosis as shown below. Thus, it was possible to analyze the segregants and, in general, evaluate the ability of such diploids to undergo meiosis. Fifty-eight progeny from 13 different teliospores of diploid FB-D11-7 were analyzed for mating type and growth factor requirements (Table 3). The segregation of auxotrophic markers confirmed that the strain is heterozygous for both pan and ade. (Once again, the low recovery of ade⁻ segregants may be due to poor growth of these segregants.) We recovered only two mating types among the progeny (Table 3), al b1 and al b2, showing that the diploid strain

| Table 3. Analysis of meiotic segregants from a+ b+ and a= b+ diploids |
|----------------------|----------------------|----------------------|
| Mating type          | From a+ b+ (FB-D12) | From a= b+ (FB-D11-7) |
| a1 b1                | 11                   | 30                   |
| a2 b2                | 17                   | 0                    |
| a1 b2                | 19                   | 0                    |
| a2 b1                | 13                   | 28                   |

Teliospores from FB-D12 (a1/a2 b1/b2 pan⁺/+ ade⁻/+ and FB-D11-7 (a1/al b1/b2 pan⁺/+ ade⁻/+ were germinated on complete medium (11). Microcolonies from each of the teliospores were spread on complete medium to obtain purified colonies of the meiotic segregants. An average of five meiotic products from each of 13 teliospores was analyzed for mating type and auxotrophy. pan⁻ ade⁺, pan⁺ ade⁻, pan⁻ ade⁻, and pan⁺ ade⁻ segregants were 32, 5, 6, 17, respectively, for FB-D12, and 17, 8, 5, 16, respectively, for FB-D11-7.

| Table 4. Tumor formation, teliospore production, and filamentous growth by different diploid strains |
|---------------------------------------------------------------|---------------|---------------|---------------|
| Diploid strain (no. of analyzed)                             | Tum | Tel | Fuz |
| a+ b+ (2)                                                     | +   | +   | +   |
| a= b+ (3)                                                     | -   | -   | -   |
| a= b+ (7)                                                     | +   | +   | -   |

Tum, tumor formation; Tel, teliospore production; Fuz, filamentous growth. Diploid strains analyzed were listed in Table 1. Plants were inoculated with ~0.3 ml of a culture of the different diploids at 10⁶ cells per ml. Symptom development was followed for 1 month under growth conditions described in Materials and Methods. Three or four plants were inoculated for each diploid strain.
FB-D11-7 is heterozygous for \( b \) and either homozygous or hemizygous for \( a \). Teliospore germination and meiosis by this \( a = b^+ \) diploid would be indistinguishable from that by \( a^+ b^+ \) diploids, indicating that \( a^+ \) is not necessary for either process.

**DISCUSSION**

We have constructed a set of diploid strains that has allowed us to determine the requirement for \( a^+ \) in filamentous growth and to confirm the requirement for \( b^+ \) in this process and tumor formation in \( U. \ maydis \). First, we obtained a diploid heterozygous at both \( a \) and \( b \) (\( aI/a2 bI/b2 \)). This diploid exhibits filamentous growth (Fuz\(^-\)) on charcoal medium and is solopathogenic. From this diploid we obtained derivatives, after UV irradiation, that do not exhibit filamentous growth (Fuz\(^-\)). These are assumed to be either homozygous (such as \( bI/bI \)) resulting from mitotic recombination or, less likely, hemizygous (such as \( bI/- \)) resulting from chromosome loss. These Fuz\(^-\) derivatives are of four types, inferred from their mating reaction with a set of haploid tester strains (Fig. 1; Table 2): \( aI/a2 bI/b1, aI/a2 b2/b2, aI/bI/b2, \) and \( a2/a2 bI/b2 \). We were able to confirm the genotypes of the latter two types because they undergo meiosis.

Analysis of these Fuz\(^-\) diploids has led to four conclusions: (i) \( a^+ \) is necessary for maintenance of filamentous growth; (ii) \( a^+ \) is not required for pathogenicity; (iii) \( a^+ b^+ \) does not block mating; and (iv) \( a^+ \) is required neither for teliospore germination nor for meiosis. Although these findings have been made for diploids, we consider it only a modest extrapolation to propose that the dikaryon has the same genetic requirements for filamentous growth and pathogenicity.

**Possible Roles of \( a \) and \( b \) in Filamentous Growth.** The requirement of \( a^+ \), in addition to \( b^+ \), for maintenance of the filamentous state places \( U. \ maydis \) nicely in line with other basidiomycetes such as *Schizopyllum commune* and *Coprinus cinereus*, which also require different alleles at two loci for maintenance of the dikaryotic state.

Although the cytology and morphology of the *U. maydis* filamentous dikaryon are not well defined (14), these are much better understood in *S. commune* and *C. cinereus*. This provides a framework for thinking about the specific roles of the \( a \) and \( b \) loci of *U. maydis*. We review briefly some of the steps in the development of the dikaryon of these basidiomycetes and the involvement of their incompatibility loci in these processes.

Multiple alleles at two loci, \( A \) and \( B \), govern establishment and maintenance of the dikaryon in *S. commune* (15) and *C. cinereus* (16). The steps leading to establishment include fusion of cells from two haploid filaments, reciprocal exchange of nuclei, and migration of the donor nucleus through the septated recipient filament until it reaches the tip cell, where it pairs with the resident nucleus to establish the dikaryotic state. A special mechanism has evolved for maintenance of the dikaryon: the nuclei divide in a coordinated fashion, and sister nuclei become partitioned to daughter cells in the growing hyphal tip via a characteristic structure, the clamp connection. The process of coordinate division and partition of nuclei via clamp connections is repeated each time the tip cell divides. This process ensures maintenance of dikaryosis as the filament grows. Some of these steps in the maintenance phase are controlled by \( A \) and others are controlled by \( B \).

Whether *U. maydis* dikaryons possess clamp connections is not known (14), but some kind of mechanism must exist to ensure proper distribution of the two genetically distinct nuclei. By analogy with the other basidiomycetes, we can imagine several types of processes that might be controlled by \( a \) and \( b \). For example, they might govern proper distribution of nuclei; by controlling the coordinate duplication of nuclei, by influencing the alignment of the nuclei, or by directing their proper movement via clamp connections. Perhaps \( a \) and \( b \) encode proteins of the nuclear membrane responsible for nucleus–nucleus recognition or for association with the cytoskeleton. Another possible role for these loci is that they govern tip cell growth by controlling, for example, membrane deposition (vesicle movement, cytoskeletal organization, etc.).

**Heterozygosity at \( a \) or \( b \) Does Not Block Mating.** Our results show that \( a^+ b^- \) and \( a^+ b^- \) strains are capable of mating. Thus, we conclude that the presence of different alleles at \( a \) or \( b \) does not block the mating reaction. Similar observations have been made in *S. commune* (17, 18) and *C. cinereus* (19, 20) and for other filamentous fungi (ref. 21; O. Yoder, personal communication). This contrasts with the ascomycetous yeast *Saccharomyces cerevisiae*, where the presence of different alleles of the mating type locus prevents mating (22).

Although yeast and these filamentous fungi differ in this regard, they both carefully control the ploidy of the cell that shall undergo meiosis. For yeast, cell fusion is followed immediately by karyogamy, to produce a diploid cell that is able to undergo meiosis. For filamentous fungi, such as the basidiomycetes described here, karyogamy does not follow cell fusion: a dikaryon is produced. Formation of a diploid occurs later in the life cycle. The restriction of ploidy in yeast occurs by governing interactions between cells (23); we imagine that the restriction in filamentous fungi described above occurs by regulating interactions between nuclei. This gives further fuel to the speculation that \( a \) and \( b \) may encode or control nuclear membrane proteins.

**Pathogenicity of Fuz\(^-\) Diploids and Implications for Pathogenicity of Dikaryons.** Coniofication experiments, in which plants are inoculated with a mixture of haploids, show that tumor formation occurs only if the haploids differ at both \( a \) and \( b \) (5–7, 9, 24). As we have discussed above for filamentous growth, the requirements for different alleles of the mating type loci in maintenance of tumor-inducing ability can be rigorously assessed by using diploids. Our analysis of tumor induction by Fuz\(^-\) diploids affirms that heterozygosity at \( b \) is required for pathogenicity and demonstrates that heterozygosity at \( a \) is not. Diploids homozygous for \( b \) are not pathogenic (refs. 5 and 10; this work), but they can induce tumors when inoculated with strains carrying a different \( b \) allele (ref. 5; F.B., unpublished data). In contrast, we have observed that \( a^+ b^+ \) diploids are pathogenic and induce the formation of tumors that appear indistinguishable from those of the parental diploid (\( a^+ b^+ \)) under conditions of high- or low-density inoculation. This result clearly demonstrates that \( a \) is not required for pathogenesis once strains have fused to form a stable diploid with the appropriate information at \( b \) (\( b^- \)). Our studies with the \( a^+ b^- \) Fuz\(^-\) diploids thus document and substantiate earlier claims (6, 9) that \( a^+ \) is not necessary for pathogenicity of a diploid.

The behavior of \( a = b^- \) strains raises the question of the necessity of the filamentous state for tumor induction. The general correlation is that the strains that give a positive fuzz reaction are tumor inducing. However, \( a = b^- \) strains are Fuz\(^-\), but still tumor inducing. Other situations (natural isolates, mutants) in which fuzz and pathogenicity are not correlated have been observed (ref. 6; F.B., unpublished data). The ability of the \( a = b^- \) strain to induce tumors might indicate that hyphae are not essential for tumor formation or that the incipient hyphae formed by these strains suffice. Another possibility is that hyphae are essential for tumor formation and that the plant provides a factor allowing their development. It is not known whether \( a = b^- \) diploids form hyphae in planta.

We conclude by summarizing the sequence of events that occurs upon coniofication with haploid strains differing at both
loki (for example, a1 b1 and a2 b2): cell fusion takes place, perhaps promoted by a or b, to form a dikaryotic cell (establishment of dikaryosis). The a and b loci then trigger a new developmental program resulting in filament formation. We have identified at least two genes distinct from a and b that are necessary for formation of filaments and that may be regulated in some manner by a and b (F.B., unpublished observations). Maintenance of the dikaryotic state and filamentous growth are governed by both a and b, as in S. commune. The filamentous dikaryon is then competent to induce tumor formation under control of the b locus. Understanding how a and b control filamentous growth and tumorigenicity will require understanding the structure of these loci and identification and analysis of other genes necessary for these processes.

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