*Proc. Natl. Acad. Sci. USA*
Vol. 84, pp. 4161–4165, June 1987
Genetics

# Genetic transformation of the fungal pathogen responsible for rice blast disease

(plant pathology/*Pyricularia/Magnaporthe grisea*)

KENNETH A. PARSONS*, FORREST G. CHUMLEY, AND BARBARA VALENT

E. I. du Pont de Nemours & Company, Central Research and Development Department, Experimental Station, E402/2208, Wilmington, DE 19898

*Communicated by Charles J. Arntzen, February 13, 1987 (received for review November 5, 1986)*

ABSTRACT **The analysis of complex genetic determinants that control the ability of a fungus to colonize its host has been impaired by the lack of sophisticated genetic tools for characterizing important pathogens. We have developed a system for the genetic transformation of *Magnaporthe grisea*, the causal agent of rice blast disease, to overcome this limitation. A *M. grisea* arginine auxotroph was shown to contain a mutation (*arg3-12*) that abolishes ornithine carbamoyltransferase activity. *M. grisea* strains that contain *arg3-12* were used as recipients in transformation experiments with plasmid pMA2, which carries the *ArgB*$^+$ gene from *Aspergillus nidulans*. Stable prototrophic transformants arose at a frequency of about 35 per microgram of plasmid DNA. Integration of single or multiple plasmid copies occurred at a single site in the genome of each transformant; rearrangements were often created during integration. When *M. grisea* genomic segments were incorporated into pMA2, the presence of any one of five different *M. grisea* segments did not greatly affect the efficiency of transformation. Integration via homologous recombination occurred when the donor plasmid was linearized by cleaving at a unique restriction site within the *M. grisea* segment.**

*Magnaporthe grisea* (1, 2) (anamorph, *Pyricularia oryzae* Cav. or *P. grisea*) is a fungal pathogen of many grass species, although the host range of any individual fungal isolate is usually narrow. The fungus is the cause of rice blast, the most widely distributed and most damaging disease of rice. Efforts to control this disease by breeding resistant rice cultivars have been frustrated by the variability of the pathogen. Hundreds of races have been distinguished among *M. grisea* rice pathogens according to the spectrum of rice cultivars they can infect. *M. grisea* is a filamentous heterothallic Ascomycete with septate hyphae that contain a single nucleus per cell. The fungus grows well on defined media; auxotrophic and morphological mutants are easily obtained (3). Genetic differences between strains can be analyzed through sexual crosses. *M. grisea* is thus an important plant pathogen that provides a model system for the genetic and biochemical analysis of many features of pathogenicity and host specificity.

Developing a well-defined, efficient system for the introduction of DNA sequences into *M. grisea* will facilitate the cloning of *M. grisea* genes that govern features of pathogenicity and host specificity. Transformation systems for a number of Ascomycetes have been described. These systems allow the transformation of *Saccharomyces cerevisiae* (4), *Neurospora crassa* (5), and *Aspergillus nidulans* (6) among others (7). This paper describes experiments in which we have obtained stable transformants of *M. grisea*; these experiments represent a first step toward the molecular characterization of pathogenicity determinants in this fungus.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

## MATERIALS AND METHODS

**Materials.** Argininosuccinate, L-arginine, 2,3-butanedione monoxime, L-ornithine, L-citrulline, diphenylamine-4-sulfonic acid (grade I), Trizma base, Trizma hydrochloride, D-sorbitol, Triton X-100, Tween 20, dextran sulfate, and ampicillin were purchased from Sigma; $MgSO_4$, D-fructose, dextrose, sucrose, sea sand, 2-propanol, Bacto-agar, Bacto-yeast extract, Bacto-Casamino acids, from Fisher; ultrapure CsCl, electrophoresis grade agarose, $NaDodSO_4$, all restriction enzymes, and bacteriophage λ DNA, from Bethesda Research Laboratories; GeneScreen*Plus* and [$\alpha$-$^{32}$P]dCTP, from New England Nuclear; polyethylene glycol 4000, from BDH; Zymolyase 60,000 (lot 2830707), from Kirin Brewery (Tokyo); and formamide, from J. T. Baker. Novozym 234 (batch PPM-1530) was generously supplied by Novo Laboratories (Wilton, CT).

**Plasmids.** Plasmid pMA2 was supplied by W. Timberlake (University of California, Davis). The plasmid has an *Xba* I fragment containing the *A. nidulans ArgB*$^+$ gene (8) blunt-end ligated into the *Pvu* II site of pBR329 (9). Plasmids pCB550, pCB551, pCB552, pCB553, and pCB566 were created by the insertion of random *Bam*HI fragments from the genome of *M. grisea* strain CP143 into the *Bam*HI site of pMA2. The sizes of the fragments are as shown in Fig. 1.

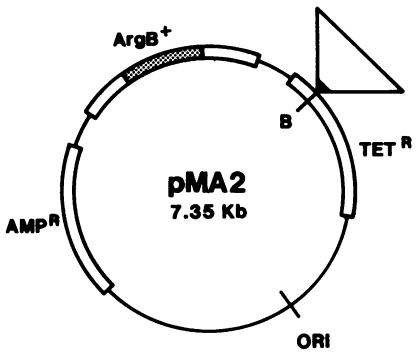
**Strains.** *M. grisea* strains used were CP422 (*mat1-2 arg3-12*), a UV-induced ornithine carbamoyltransferase (OrnCbmTase; carbamoyl phosphate:L-ornithine carbamoyltransferase, EC 2.1.3.3)-deficient mutant derived from strain 4091-5-8, and 4170-1-3 (*mat1-1 arg3-12*), produced by crossing CP422 with 4136-1-2 (*mat1-1*), a wild-type laboratory strain. Strain 4091-5-8 (*mat1-2*) is a pathogen of both weeping lovegrass and goosegrass. The induction of mutations in *M. grisea* was described by Crawford *et al.* (3). Strains CP521, CP522, CP525, CP531, CP532, CP533, and CP534 were produced by transformation of CP422 with pMA2. Strains CP609, CP610, CP611, CP612, and CP613 were produced by transformation of 4170-1-3 with circular pCB550. Strains CP537, CP541, CP542, and CP543 were produced by transformation of CP422 with circular pCB550. Strains CP618, CP619, CP621, and CP622 were produced by transformation of 4170-1-3 with *Kpn* I-cleaved pCB550. Strain CP143 (*mat1-2*) is a prototrophic laboratory strain pathogenic to rice.

*Escherichia coli* strain DB6507 (*pyrF*::Tn*5 strA recA*$^-$ *hsr*$^-$ *hsm*$^-$ *thr*$^-$ *leuB*$^-$ *proAB*$^-$; from D. Botstein, Massachusetts Institute of Technology) was used for all recombinant DNA manipulations.

**Enzyme Assays for OrnCbmTase Activity.** *M. grisea* cultures were grown in liquid minimal medium supplemented with arginine. Hyphae were macerated by pulse-blending 18 hr before a sample was prepared for the assay. Mycelia were collected by centrifugation and hyphae were permeabilized

Abbreviation: OrnCbmTase, ornithine carbamoyltransferase.

*Academic address: Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309.




| Plasmid | Insert size |
|---|---|
| pCB550 | 3.7 Kb |
| pCB551 | 5.4 Kb |
| pCB552 | 5.7 Kb |
| pCB553 | 5.2 Kb |
| pCB566 | 5.0 Kb |

FIG. 1. Map of plasmid pMA2 and plasmids derived from pMA2. Plasmids pCB550–pCB553 and pCB566 were created by the insertion of random *Bam*HI fragments from the genome of *M. grisea* strain CP143 into the *Bam*HI (B) site of pMA2. $AMP^R$ and $TET^R$, genes conferring resistance to ampicillin and tetracycline, respectively; ORI, origin of replication; kb, kilobases.

by the methods of Basabe *et al.* (10). Permeabilized hyphae were incubated in a Tris/acetate-buffered reaction mixture containing ornithine and carbamoyl phosphate, as described by Davis *et al.* (11). The hyphae were pelleted by centrifugation and the supernatant was assayed for the presence of citrulline by the method of Koritz and Cohen (12).

**Reversion Frequency of CP422.** CP422 was grown on supplemented oatmeal agar plates for about 4 weeks. Liquid minimal medium containing 0.05% Tween 20 detergent was added to the plates, and conidia were scraped from the fungal mass with a flame-sterilized glass spreader. The titer of the suspension was determined by counting using a hemocytometer. Conidia were spread onto sorbose complete medium to determine viability. Conidia were spread onto minimal agar plates to determine the reversion frequency.

**Transformation of *E. coli*.** The transformation methods used were described by Davis *et al.* (13).

**Transformation of *M. grisea*.** Transformation proceeded according to the methods of Hinnen *et al.* (4) as modified by Yelton *et al.* (6). Protoplasts were prepared from fungus grown in liquid culture. Cultures were expanded by periodic maceration with a Waring Blendor. Blending breaks the fungal hyphae into fragments of a few cells, creating a more uniform culture with a shorter doubling time. Cultures were last blended 16–18 hr before protoplast preparation. Protoplasts were released enzymatically by treatment with Novozym 234 and Zymolyase 60,000 or with Novozym 234 alone.

**DNA Isolation.** *M. grisea* genomic DNA was prepared either by the method of Garber and Yoder (14) or by the method of Akins and Lambowitz (15). The procedures described by Akins and Lambowitz yielded about 10-fold more DNA than did those of Garber and Yoder. Plasmid DNA was isolated from *E. coli* by the method of Davis *et al.* (13).

**Hybridization Analysis.** Restriction digests of *M. grisea* genomic DNA were blotted to GeneScreen*Plus* by the method of Southern (16). Hybridization proceeded at 42°C in 50% formamide/1% $NaDodSO_4$/1 M NaCl/10% dextran sulfate containing calf thymus DNA at 100 μg/ml, with labeled probe DNA added to $2 \times 10^6$ dpm/ml. Plasmid DNA was radioactively labeled by the incorporation of [α-$^{32}$P]-dCTP according to the method of Rigby *et al.* (17). Filters were washed twice for 5 min at room temperature in 2× SSC (standard saline citrate; 1× is 0.15 M NaCl/15 mM sodium citrate, pH 7.0), twice for 30 min at 65°C in 2× SSC/1% $NaDodSO_4$, and twice for 30 min at room temperature in 0.1× SSC.

**Genetic Techniques.** Genetic techniques involving *M. grisea* have been described (3).

**Media.** Preparation of oatmeal, minimal, and sorbose complete media was described by Crawford *et al.* (3). Regeneration medium was described by Yelton *et al.* (6).

## RESULTS

**Analysis of Potential Recipients.** The *A. nidulans ArgB*$^+$ gene is expressed in *S. cerevisiae* (8) and in *N. crassa* (18). We reasoned that it might also complement OrnCbmTase-deficient mutants of *M. grisea*. Therefore, we set out to identify a suitable OrnCbmTase-deficient *M. grisea* recipient for transformation experiments using the *ArgB*$^+$ gene as donor DNA. Our collection of UV-induced auxotrophic mutants includes 12 that require arginine. Feeding tests were used to determine the point in the arginine biosynthetic pathway that is disrupted in each mutant.

Small hyphal plugs of the $Arg^-$ mutants were placed radially from the center of plates of minimal agar medium. Approximately 10 μg of either arginine, argininosuccinate, citrulline, or ornithine was placed in the center of the plates. As the supplements diffused toward the perimeter of the plates, the growth response of the fungal plugs was observed. Results are presented in Table 1. Eight of the 12 strains tested responded to arginine or citrulline, but not to ornithine.

In *Neurospora*, arginine auxotrophs that respond to citrulline but not to ornithine contain mutations in any one of three genes: *arg-2* or *arg-3*, which encode the two polypeptides that make up arginine-specific carbamoyl phosphate synthetase, or *arg-12*, which encodes OrnCbmTase. Enzyme assays for OrnCbmTase activity were used to determine whether any of the eight *M. grisea* strains were bona fide OrnCbmTase mutants. Table 2 presents some of the results of these assays. Only strain CP422, with the *arg3-12* mutation, lacked OrnCbmTase activity. The reversion frequency of *arg3-12* was determined to be $3.7 \times 10^{-7}$ per viable conidium.

**Transformation with Circular pMA2.** Covalently closed circular pMA2 DNA (Fig. 1) was used as the donor plasmid in transformation experiments with the OrnCbmTase-deficient *M. grisea* strain, CP422, as the recipient. Transformants arose at a frequency of about 35 per microgram of DNA, or $1.6 \times 10^{-3}$ per viable protoplast. Total genomic DNA was isolated from seven randomly chosen transformants. When undigested DNA from these transformants was electrophoresed in agarose gels, no fast-moving bands were observed, suggesting that donor plasmid DNA had integrated into sites in recipient chromosomes. Southern hybridization analysis

Table 1. Growth response of arginine auxotrophs

| Mutant allele | Substrate tested | | | | |
|---|---|---|---|---|---|
| | Min | Orn | Cit | Arg(Suc) | Arg |
| *ARG*$^+$ | + | + | + | + | + |
| *arg-1, -3, -4, -5, -6, -7, -9, -12* | − | − | + | ± | + |
| *arg-2, -8, -10, -11* | − | + | + | ± | + |

Arginine auxotrophs carrying the mutations indicated were tested for response to arginine biosynthetic intermediates as described in *Materials and Methods*. Growth (+), failure to grow (−), or an intermediate response (±) are indicated. Min, unsupplemented minimal medium; Orn, ornithine; Cit, citrulline; Arg(Suc), argininosuccinate; Arg, arginine. The wild-type *ARG*$^+$ strain used was 4091-5-8. The *arg*$^-$ mutants were described by Crawford *et al.* (3).

Table 2. Enzyme assays of an OrnCbmTase-deficient mutant

| Strain | OrnCbmTase activity | | | |
|---|---|---|---|---|
| | Trial 1 | | Trial 2 | |
| 4091-5-8 (*ARG*$^+$) | 4.96 | (1.0) | 7.88 | (1.0) |
| Boiled 4091-5-8 | 0.12 | (0.024) | 0.0 | (0.0) |
| CP422 (*arg3-12*) | 0.062 | (0.0125) | −0.018 | (0.0) |
| Boiled CP422 | −0.041 | (0.0) | 0.0 | (0.0) |

OrnCbmTase activity is reported as μmol of citrulline produced per min per mg (dry weight) of mycelia. Numbers in parentheses have been standardized to 4091-5-8 in each trial. Lines 2 and 4 show the results obtained when the permeabilized hyphae were boiled before OrnCbmTase activity was assayed.

of the undigested DNA from the seven transformants revealed pMA2 homology in the high molecular weight genomic DNA (data not shown). DNA from the seven transformants was then digested with either *Bam*HI, *Kpn* I, or *Xho* I, electrophoresed, blotted, and probed with $^{32}$P-labeled pMA2 DNA.

Southern analysis of transformant DNA digested with restriction endonucleases that do not cut within pMA2 (*Kpn* I or *Xho* I) revealed that plasmid DNA was integrated at only one genomic site in each transformant (data not shown). Southern blots of transformant DNA digested with *Bam*HI, an enzyme that cuts once within pMA2, revealed more about the nature of the integration events (Fig. 2). Of the seven transformants analyzed, one (CP525) is thought to have been produced by the integration of a single unrearranged copy of pMA2 into the genome. The other six have multiple copies in tandem, based on the intensity and the pattern of the bands on the autoradiogram. At least three of the transformants with tandem arrays (CP532, CP533, and CP534) experienced complex plasmid rearrangements coincident with the integration event. When pBR322 was used as a probe, instead of pMA2, the same patterns were observed (data not shown). The results shown in Fig. 2 are consistent with the conclusion that plasmid DNA has integrated at a different site in each of the transformants examined. No homology to pBR322 or pMA2 was observed in the genome of the untransformed recipient strain under the hybridization conditions used throughout these experiments.

All seven transformants were crossed both to wild-type strains and to strain 4170-1-3 (*mat1-1 arg3-12*). Analysis of the progeny of these crosses confirmed that each transformant had the *ArgB*$^+$ gene integrated at a single site in its genome and that the sites were not linked to the resident OrnCbmTase-encoding gene. A Southern blot of the ascospore progeny from a single tetrad from the cross CP533 × 4170-1-3 is shown in Fig. 3. The cosegregation of the Arg$^+$ phenotype with the *ArgB*$^+$ pMA2 sequence homology is confirmed. Results presented in Fig. 3 show that the complex pattern of bands homologous to pMA2 segregates like a simple Mendelian determinant. This observation further strengthens the conclusion that plasmid DNA is integrated at a single site in the genome of the Arg$^+$ transformant CP533.

**Transformation with pMA2 Derivatives That Carry *M. grisea* Sequences.** Plasmid pMA2 carries no *M. grisea* genomic DNA segments. Five derivatives of pMA2 (pCB550, pCB551, pCB552, pCB553, and pCB566) were constructed by inserting random *Bam*HI fragments from CP143 genomic DNA into the single *Bam*HI site on the plasmid (see Fig. 1).

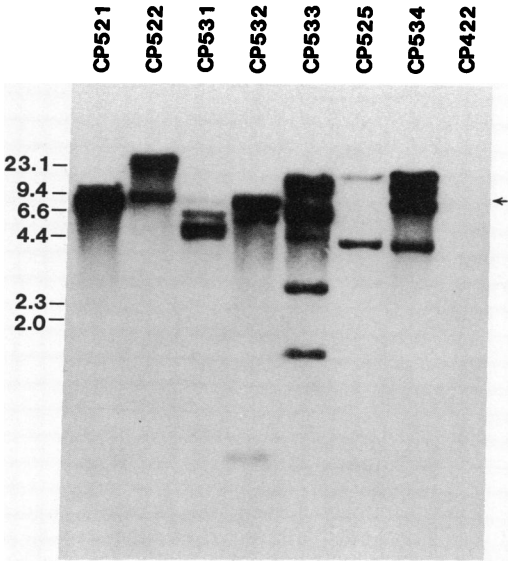



FIG. 2. Southern hybridization analysis of Arg$^+$ transformants generated with donor plasmid pMA2. The seven leftmost tracks contained genomic DNA from independent transformants (CP521, CP522, CP531, CP532, CP533, CP525, and CP534), digested to completion with *Bam*HI and hybridized with $^{32}$P-labeled pMA2 DNA. The track at extreme right contained *Bam*HI-digested DNA from the *arg*$^-$ recipient strain, CP422. In this and other figures, the positions and sizes (in kb) of bacteriophage λ DNA *Hin*dIII fragments used as standards are given at left. Arrow at right indicates position of unit-length 7.35-kb *Bam*HI-digested pMA2 DNA. Since *Bam*HI cuts once in pMA2, two junction fragment bands should be revealed for each site of plasmid integration in transformant DNA. A band the size of unit-length plasmid will also be seen, if a transformant contains pMA2 sequences in an unrearranged tandem array. Transformant CP525 may contain a single integrated copy of pMA2. The other six transformants all contain tandemly arrayed copies of pMA2, with CP531, CP533, and CP534 representing cases where sequence rearrangements have occurred.

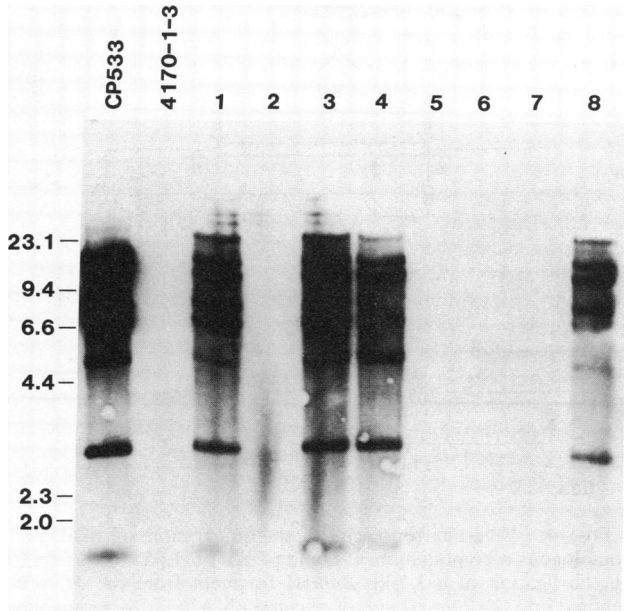



FIG. 3. Mendelian transmission of a rearranged tandem array of integrated pMA2 sequences. Arg$^+$ transformant CP533 (which contains a number of rearranged copies of donor plasmid pMA2 in a tandem array) was crossed with strain 4170-1-3 (which contains the OrnCbmTase mutation *arg3-12*). Southern hybridization analysis of the parents and each of eight ascospore progeny from a single tetrad is presented. Genomic DNA from each strain was digested with *Bam*HI and hybridized with $^{32}$P-labeled pMA2 DNA. Ascospores 1, 3, 4, and 8 are Arg$^+$; ascospores 2, 5, 6, and 7 are Arg$^-$. Analysis of parental CP533 DNA revealed at least eight hybridizing bands in a range of sizes from about 23 kb to less than 1 kb. The Arg$^+$ ascospores all contained the same pattern of hybridizing bands as their CP533 parent, while the Arg$^-$ ascospores showed no homology to the probe.

When any of these plasmids was used as donor DNA in transformation of strains that carry *arg3-12*, there was no increase in the efficiency of transformation over that observed with pMA2. One of the plasmids, pCB566, carries a sequence or sequences found in many copies in the CP143 genome (F.G.C., unpublished results). The efficiency of transformation was decreased when plasmid pCB552 was used, falling by a factor of about 25, to approximately 1.4 transformants per microgram of plasmid DNA, or $6.5 \times 10^{-5}$ per viable protoplast.

Southern hybridization analysis of nine transformants generated by using plasmid pCB550 showed that nonhomologous integration occurred when the plasmid was used in covalently closed circular form (data not shown; see Fig. 4). Genetic analysis also showed that the *M. grisea* genomic segment carried by pCB550 did not target the site of plasmid integration. A total of eight independent transformants, four of each mating type, were mated in all possible combinations, and progeny were analyzed for arginine auxotrophy. From all 16 of these crosses, $Arg^-$ progeny arose frequently, in a ratio of about 3 $Arg^+$ ascospores to 1 $Arg^-$ ascospore. If the transformed $ArgB^+$ gene had integrated at the same site in any two transformants, then $Arg^-$ progeny should have been rare.

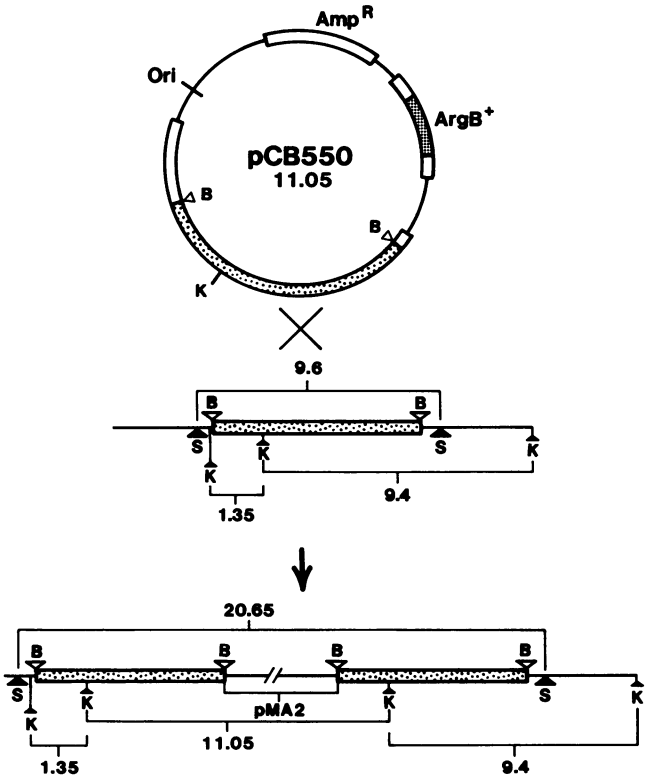



FIG. 4. Diagram representing the integration of pCB550 by homologous recombination. Plasmid pCB550 (11.05 kb) was created by the ligation of a 3.7-kb *Bam*HI fragment from the *M. grisea* genome into the *Bam*HI site of plasmid pMA2. The diagram shows a single crossover between the plasmid-borne 3.7-kb segment (stippled bar) and its homologue in the genome, leading to the integration of pMA2 sequences between copies of the 3.7-kb segment in direct repeat. In the *M. grisea* genome, the 3.7-kb *Bam*HI fragment is contained within a 9.6-kb *Sma* I fragment. The 3.7-kb *Bam*HI fragment contains a single *Kpn* I cleavage site; junction fragments of 1.35 kb and 9.4 kb are revealed when Southern blots of *Kpn* I-digested *M. grisea* DNA are probed with the 3.7-kb *Bam*HI fragment. Since pMA2 contains no *Sma* I site, integration of plasmid pCB550 by homologous recombination results in the loss of a 9.6-kb *Sma* I fragment and the appearance of a new *Sma* I fragment of 20.65 kb, or larger, if more than one copy of the plasmid is integrated. Homologous integration of pCB550 leaves the 1.35-kb and 9.4-kb *Kpn* I junction fragments unchanged, but a new 11.05-kb *Kpn* I fragment equivalent to pCB550 appears in digests of genomic DNA.

**Transformation with Linear pCB550 DNA.** We observed a markedly different result when donor pCB550 DNA was first linearized by cleavage at a unique *Kpn* I site within the *M. grisea* genomic segment and then used in transformation. Although the frequency of transformation was not affected, four of eight transformants analyzed had integrated pCB550 via homologous recombination (Fig. 4). Fig. 5 shows the results of Southern hybridization analysis of four transformants, two of which (CP618 and CP622) appear to have undergone plasmid integration via a crossover between the *M. grisea* segment carried by pCB550 and its homologue in the recipient genome. One transformant (CP622) also experienced rearrangement of a flanking genomic segment. Results similar to those shown in Fig. 5 were observed in Southern hybridization analysis of four other transformants generated with linear pCB550 DNA (data not shown).

**Recovery of Plasmid DNA and Eviction of Genomic Segments Adjacent to the Site of Integration.** Chromosomal DNA prepared from transformant CP618 (Fig. 5) was partially digested with *Kpn* I, extracted with phenol and chloroform, ligated, and used to transform *E. coli*. Ampicillin-resistant

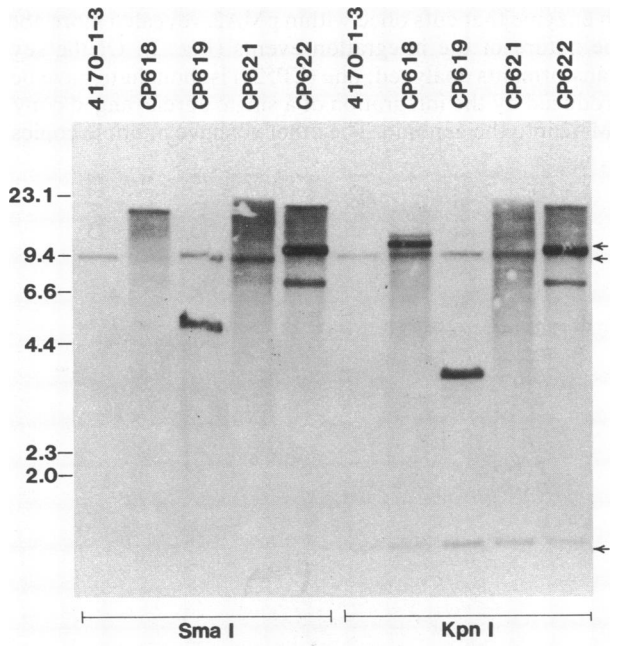



FIG. 5. Southern hybridization analysis of $Arg^+$ transformants generated with linear pCB550. *M. grisea* strain 4170-1-3 (*arg3-12*) was used as a recipient in transformation with donor pCB550 DNA that had been linearized by cleavage at the *Kpn* I site, as described in the text and in Fig. 4. Genomic DNA was extracted from the untransformed recipient and from four $Arg^+$ transformants (CP618, CP619, CP621, and CP622), digested to completion either with *Sma* I or with *Kpn* I, and hybridized with $^{32}P$-labeled pCB550 DNA. In transformant CP618, pCB550 DNA has been integrated by homologous recombination: the target 9.7-kb *Sma* I fragment present in the recipient has been replaced by a fragment of the expected 20-kb size (see Fig. 4), and analysis of *Kpn* I-digested DNA reveals the expected bands at 11 kb, 9.4 kb, and 1.4 kb (positions indicated by arrows at right). Transformant CP622 also appears to have integrated pCB550 at the target sequences, because the 9.7-kb *Sma* I fragment has been replaced by new bands at 23 kb, 10 kb, and 7.5 kb. This multiplicity of *Sma* I bands indicates that rearrangement of the target sequences also occurred in CP622, a conclusion supported by the results presented for *Kpn* I-digested DNA. Nonhomologous recombination leading to plasmid integration apparently occurred in transformant CP619. The basis for the transformed phenotype of CP621 is not clear from the results presented here.

colonies were recovered at a frequency of less than 10 per microgram of donor DNA. Plasmid DNA was isolated from 10 independent bacterial transformants and digested with *Kpn* I alone, *Bam*HI alone, or both *Kpn* I and *Bam*HI. The restriction patterns of 4 of the 10 plasmids were consistent with recovery of the original pCB550 sequences alone. The restriction patterns of the other 6 plasmids were consistent with recovery of pCB550 together with one or more of the *Kpn* I genomic fragments that flank the integration site (Figs. 4 and 5).

## DISCUSSION

The results presented above demonstrate that stable $Arg^+$ transformants can be generated by using *M. grisea* recipient strains that contain the *arg3-12* mutation, which abolishes OrnCbmTase activity, and donor plasmids that carry the *A. nidulans* $ArgB^+$ gene, which encodes OrnCbmTase. All the transformants we analyzed contain donor plasmid DNA integrated into the recipient's genome. The transformation methods we used are essentially the same as those described for transformation of *A. nidulans* (6). Transformants appeared at a frequency that is roughly equivalent to that observed in similar experiments with other fungi (4–7).

The *M. grisea* transformants we analyzed contain donor plasmid DNA integrated at no more than one site in the genome of each transformant. When plasmid pMA2 (which cannot be expected to replicate autonomously in *M. grisea*, and which carries no *M. grisea* genomic segments) was used as donor DNA, integration occurred at sites other than the resident $ARG3^+$ gene. Although a single integrated copy of pMA2 apparently confers an $Arg^+$ phenotype on transformants CP525 and CP618, most of the transformants we analyzed contain multiple copies of pMA2 in tandem array, often with complex sequence rearrangements.

Plasmid integration in transformation of *S. cerevisiae* is rarely accompanied by the formation of tandem arrays; sequence rearrangements of the type described here are not observed (4). Moreover, yeast transformation via plasmid integration depends absolutely on homologous recombination between plasmid-borne and chromosomal sequences. However, yeast appears to be exceptional among eukaryotes in these regards. Plasmid integration through nonhomologous recombination events, often accompanied by the formation of tandem arrays with complex sequence rearrangements, has been frequently observed in transformation of Euascomycetes, including *Aspergillus* and *Neurospora* (5–7). Such events are also typical of direct DNA transfer to higher eukaryotes, including plant and animal cells (19, 20). A common mechanism may underlie such complex plasmid integration events in transformation of eukaryotes ranging from fungi to human cells.

Supplying a region of genetic homology shared by donor plasmids and the *M. grisea* genome did not greatly affect transformation efficiency, nor did it alter the nature of the plasmid integration events observed in the nine transformants we analyzed. Plasmid integration via simple homologous recombination events is a necessary prerequisite to many sophisticated genetic engineering manipulations, including the eviction and transplacement of mutant genes (21). We observed homologous integration only in transformation experiments where donor plasmid DNA had previously been linearized by cleavage at a unique site in a genomic DNA segment. This result is consistent with the creation of "recombinogenic ends" in the donor molecule, as first described for yeast by Orr-Weaver *et al.* (22), and it provides a hopeful indication of the potential for genetic engineering of *M. grisea* through recombinant DNA techniques.

The results reported in this paper have encouraged us to proceed with efforts to clone *M. grisea* genes by transforming appropriate *M. grisea* recipients with gene libraries built in vectors that carry the *Aspergillus* $ArgB^+$ gene. Gene cloning systems based on transformation with $ArgB^+$ should serve in the isolation of many genes that govern general pathogenicity and host-specificity phenotypes in this ubiquitous plant pathogen.

We thank Charles Arntzen for a valuable critical reading of this manuscript, and we thank Doreen Lewandowski for patient, expert assistance in preparing the manuscript.

1. International Rice Research Institute (1965) *The Rice Blast Disease: Proceedings of a Symposium at the International Rice Research Institute* (Johns Hopkins Univ. Press, Baltimore, MD), p. 512.
2. Barr, M. E. (1977) *Mycologia* **69,** 952–966.
3. Crawford, M. S., Chumley, F. G., Weaver, C. G. & Valent, B. (1986) *Genetics* **114,** 1111–1129.
4. Hinnen, A., Hicks, J. B. & Fink, G. R. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1929–1933.
5. Case, M. E., Schweizer, M., Kushner, S. R. & Giles, N. H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5259–5263.
6. Yelton, M. M., Hamer, J. E. & Timberlake, W. E. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1470–1474.
7. Hynes, M. J. (1986) *Exp. Mycol.* **10,** 1–8.
8. Berse, B., Dmochowska, A., Skrzypek, M., Weglenski, P., Bates, M. A. & Weiss, R. L. (1983) *Gene* **25,** 109–117.
9. Boylan, M., Holland, M. J. & Timberlake, W. E. (1986) *Mol. Cell. Biol.* **6,** 3621–3625.
10. Basabe, J. R., Lee, C. A. & Weiss, R. (1979) *Anal. Biochem.* **92,** 356–360.
11. Davis, R. H., Ristow, J. L. & Ginsburgh, C. L. (1981) *Mol. Gen. Genet.* **181,** 215–221.
12. Koritz, S. B. & Cohen, P. P. (1954) *J. Biol. Chem.* **209,** 145–150.
13. Davis, R. W., Botstein, D. & Roth, J. R. (1980) *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 116–117.
14. Garber, R. C. & Yoder, O. C. (1983) *Anal. Biochem.* **135,** 416–422.
15. Akins, R. A. & Lambowitz, A. M. (1985) *Mol. Cell. Biol.* **5,** 2272–2278.
16. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–507.
17. Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 737–751.
18. Weiss, R. L., Puetz, D. & Cybis, J. (1985) in *Gene Manipulations in Fungi*, eds. Bennett, J. W. & Lasure, L. L. (Academic, Orlando, FL), pp. 280–292.
19. Paszkowski, J., Shillito, R. D., Saul, M., Mandak, V., Hohn, T., Hohn, B. & Potrykus, I. (1984) *EMBO J.* **3,** 2717–2722.
20. Perucho, M., Hanhan, D. & Wigler, M. (1980) *Cell* **22,** 309–317.
21. Winston, F., Chumley, F. & Fink, G. R. (1983) *Methods Enzymol.* **101,** 211–227.
22. Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6354–6358.