APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1991, p. 2714–2723
0099-2240/91/092714-10$02.00/0

Vol. 57, No. 9

Expression of Proteins and Glycoproteins Encoded by the Haploid Nuclei in the Dikaryotic State in the Basidiomycete *Agrocybe aegerita*

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Received 29 March 1991/Accepted 11 June 1991

The total proteins and concanavalin A-binding glycoproteins of the cultivated mushroom *Agrocybe aegerita* were studied in homokaryotic siblings and in dikaryotic strains. The glycoproteins exhibited considerable variability compared with the proteins; the genetic diversity detected in homokaryons in the glycoprotein analysis was 30-fold higher than the genetic diversity revealed by protein analysis, and the glycoprotein patterns could be used to characterize individual genotypes. We found that the expression of glycoproteins in haploid nuclei was significantly asymmetric when the nuclei were paired in dikaryons. The expression levels of the two component nuclei depended on their genotypes, and each haploid nucleus was characterized by its level of expression. Furthermore, some specific glycoproteins that were not detected in all of the homokaryons were newly synthesized in the dikaryotic strains. Among these was a glycoprotein designated gpAa-65, which was identified in all of the dikaryotic strains and appeared to be a good molecular marker of the dikaryotic state.

Agrocybe aegerita, a mushroom belonging to the class Agaricales, is industrially cultivated for food in Italy and Spain. The following two mycelial forms are distinguished in this organism: the homokaryotic mycelium form containing haploid nuclei having the same genotype and the dikaryotic mycelium form containing haploid nuclei having two different genotypes. A homokaryon is nonfertile and develops upon germination from a single sexual spore, and a dikaryon is fertile and is formed by anastomosis of two compatible homokaryons which are heteroallelic for both the *A* and *B* incompatibility factors (14). This breeding system restricts self-fertilization and thus maintains genetic heterogeneity within natural populations.

In some basidiomycetes, such as *Schizophyllum commune*, each incompatibility factor is composed of two closely linked loci which are multiallelic (10, 23). These loci are involved in the genetic control of fruit body production (13, 27) and of the dikaryotic state (for a review see reference 19). Although the incompatibility system has been the subject of numerous genetic studies, the molecular bases of functional and regulatory mechanisms remain unresolved. An important problem for genetic improvement of varieties and, more particularly, for the selection of powerful homokaryotic components is the expression of the two haploid nuclei within a dikaryon. Studies on protoplast regeneration (20) have shown indirectly that there is dissymmetry in the mitotic regulation of the two nuclei, but no direct evidence for such dissymmetry in the expression of the nuclei has been obtained yet.

Most studies concerning dikaryotization have been conducted with the aim of identifying molecular markers that are specific for the dikaryotic state. Comparisons of the isozyme patterns of homokaryotic and dikaryotic strains of the basidiomycetes *Schizophyllum commune* (29) and *Coprinus congregatus* (7) revealed no isozyme differences. Other studies with *Schizophyllum commune* (5) and *Agrocybe aegerita* (25) showed that there were small differences in the patterns of protein synthesis between homokaryons and dikaryons when the organisms were grown vegetatively. Moreover, the homokaryons and dikaryons of *Schizophyllum commune* have been found to contain similar mRNA populations (9, 22), and no significant difference was detected between the two-dimensional patterns of proteins synthesized in vitro from polysomal RNAs of the two types of mycelia (9). The lack of any evidence for significant qualitative differences in the patterns of in vitro translation products led Hoge et al. (9) to conclude that posttranslational modifications may play a major role in the regulation of dikaryosis. In the myxomycete *Dictyostelium discoideum* it has been shown that glycoproteins exhibit more variability than proteins (3). Accordingly, we carried out a two-dimensional analysis of the glycoproteins of *Agrocybe aegerita* to study the expression of this class of molecules in the two-component haploid nuclei of dikaryotic strains and to characterize the dikaryotic state for specificity of glycoprotein expression.

In this study we investigated the variability of glycoproteins (defined as concanavalin A [Con A]-binding proteins) in homokaryotic progeny that came from a wild-type strain and in dikaryons that were derived from anastomosis of compatible homokaryons by using two-dimensional electrophoresis (isoelectric focusing and sodium dodecyl sulfate [SDS]-polyacrylamide gel electrophoresis [PAGE]). The genetic variability in homokaryotic and dikaryotic strains was estimated by comparing their glycoprotein patterns. This comparison was also carried out in order to assess the possibility of characterizing industrial varieties. The levels of protein expression and glycoprotein expression from each homokaryotic component in the dikaryotic strains were also studied to determine whether dissymmetric expression does exist between the two haploid nuclei of dikaryons. Proteins and glycoproteins that were specifically expressed in the dikaryotic state were identified, and protein markers of this state were sought.

MATERIALS AND METHODS

Strains. All of the homokaryotic strains used in this study were obtained from the germination of single basidiospores

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collected from mature basidiocarps of wild-type strains, as previously described (11). Homokaryotic strains F41 (*A1B1*), F24 (*A1B1*), F30 (*A1B1*), F12 (*A2B2*), F31 (*A2B2*), F9 (*A1B2*), F3 (*A1B2*), F34 (*A2B1*), F25 (*A2B1*), and F6 (*A2B1*) were derived from progeny of wild-type dikaryotic strain SM51 (25), and homokaryotic strains A17 (*A9B9*) and A117 (*A10B5*) were derived from wild-type dikaryotic strain SM871027. Dikaryotic strains F30/F31, F30/A17, F30/A117, F31/A17, F31/A117, and A17/A117 were constructed by anastomosis of four compatible homokaryons (F30, F31, A17, and A117).

Culture conditions. Mycelia were grown on solid complete Raper medium (21) in petri dishes for eight days at 25°C in the dark; the mycelia were then harvested with a scalpel (about 0.1 g [dry weight] per petri dish), resuspended in 10 ml of complete Raper liquid medium, and disrupted for 30 s at 4°C with a Polytron homogenizer (Kinematica) equipped with shaft type 10TS. The fragmented mycelia were then inoculated in 100-ml portions of complete Raper liquid medium supplemented with 50 μg of ampicillin per ml in Roux culture flasks and incubated for 140 h at 25°C in the dark. After incubation, the grown mycelia were harvested by filtration on sterilized gauze, thoroughly washed with cold distilled water, dried, and weighed. The dried mycelia (about 2 g per flask) were immediately broken for protein extraction. All of the mycelia analyzed were cultivated by using these standard conditions. The growth conditions and age of cultures at the time of harvest were particularly important for obtaining repeatable protein and glycoprotein patterns. The use of fragmented mycelia as inocula was essential because, in this case, strains had equivalent and reproducible growth rates (29). When these conditions were used, analysis of mycelia harvested at different times showed that protein and glycoprotein patterns remained reproducible when mycelia were harvested after 120 to 168 h of cultivation. Accordingly, all mycelia were harvested after 140 h of cultivation.

Protein sample preparation. For total protein extraction, 2 g of mycelium was resuspended in 1.6 ml of extraction buffer (0.05 M Tris hydrochloride [pH 6.8], 2 mM EDTA, 5 mM $MgCl_2$, 50 μg of RNase A per ml, 4.8 mM phenylmethylsulfonyl fluoride, 1% SDS) and broken up by sonication with a Vibracell VC500 sonicator as previously described (25). Proteins were solubilized by adding 1 volume of SDS sample buffer (0.1 M Tris hydrochloride, 4% SDS, 30% glycerol, 10% β-mercaptoethanol; pH 6.8). Suspensions were homogenized by vortexing, heated at 100°C for 5 min, and allowed to stand on ice for at least 5 min. They were then clarified by centrifugation at 10 000 × *g* for 15 min at 4°C. The supernatants were considered the total protein extracts.

Two-dimensional electrophoresis. Protein samples were separated in the first dimension by isoelectric focusing as described by O'Farrell (18) and in the second dimension by SDS-PAGE, using a separating gel containing a 17 to 22% exponential acrylamide gradient and a 5% acrylamide upper stacking gel as described previously (25). Gels were stained by using the ultrasensitive silver stain of Oakley et al. (17).

Protein blotting. Proteins separated by two-dimensional SDS-PAGE were blotted onto nitrocellulose membrane filters (type BA85; Schleicher & Schuell) by using the Multiphor II-Novablot electroblotting system (LKB). After electrophoresis, the two-dimensional gels were immersed in transfer buffer (39 mM glycine, 20% methanol, 48 mM Tris) for 15 min. Transfer units were assembled on the anode by stacking (from bottom to top) nine filter paper sheets, the nitrocellulose filter, the two-dimensional gel, and nine more filter paper sheets. All components of the transfer unit were prewetted with transfer buffer. For efficient transfer, a current of 0.8 mA/cm^2 of transfer unit was applied for 2 h.

Detection of Con A-binding proteins. To detect Con A-binding proteins, the nitrocellulose filters obtained after transfer of proteins were incubated for 2 h in saturation buffer (0.2 M NaCl, 0.05 M Tris hydrochloride [pH 7.4], 3% bovine serum albumin) and for 2 h in saturation buffer containing 0.5 mg of Con A per ml and 20 mM α-methylmannoside. The blots were then washed three times in TBS (0.2 M NaCl, 0.05 M Tris hydrochloride [pH 7.4]) containing 0.05% SDS and 0.2% Triton X-100 and twice in TBS. The filters were incubated in a second step with saturation buffer containing 250 U of horseradish peroxidase (grade I; Boehringer) for 2 h and then subjected to a new cycle of washes. Peroxidase binds specifically to Con A-binding proteins because Con A is a tetramer with four binding sites and peroxidase is itself a Con A-binding protein (8). For the color reaction, filters were soaked in TBS containing 15% methanol, 0.012% H_2O_2, and 1.2 mg of 4-chloro-1-naphthol per ml.

The use of α-methylmannoside at a concentration of 20 mM allowed Western blots to be obtained with a clean background without inhibiting the fixation of Con A. The stability of the glycoprotein-Con A-peroxidase complexes was tested by using increasing concentrations of α-methylmannoside (50, 100, 200, and 400 mM). Detection sensitivity was reduced with 50 mM α-methylmannoside, and detection was completely abolished with 200 mM α-methylmannoside, showing that the Con A-glycoprotein interaction was dependent on the sugar-binding site.

No change in the Con A-binding protein profiles was observed when lipids were removed from protein extracts by extraction with chloroform-ethanol (2:1, vol/vol), indicating that only glycoproteins were detected. The lack of detection of complex polysaccharides was verified by performing proteolysis experiments; no staining was observed on blots obtained from protein extracts treated with 1 mg of proteinase K per ml and then analyzed for glycoprotein detection with Con A-peroxidase. In the course of these proteolysis experiments a protein sample that was not treated with proteinase K was analyzed in parallel (i.e., on the same gel); the efficient detection of Con A-binding proteins in this sample showed that neither Con A nor peroxidase was altered by the proteinase K contained in the samples subjected to proteolysis.

Establishment and comparison of standard maps. To establish standard maps, three independent protein samples were prepared from each strain, subjected to two-dimensional SDS-PAGE, and silver stained (for standard protein maps) or transferred to nitrocellulose filters and treated with Con A-peroxidase (for standard glycoprotein maps). For proteins or glycoproteins, the three two-dimensional patterns (nitrate-stained gels or Con A-peroxidase-stained blots) obtained from the independent extracts were traced onto a transparent overlay sheet, and the diagrams were compared by superimposing the transparent sheets. For each strain, only protein and glycoprotein spots which were identified from all three samples were considered reproducible spots; nonreproducible protein or glycoprotein spots were eliminated (about 4% of the spots detected). All standard maps were constructed by using the Mac Draft drawing software. Total protein and glycoprotein maps were squared as previously described (2) by drawing vertical lines corresponding to the different pH values of the first dimension (pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5) and horizontal lines corresponding to apparent molecular weights (110,000, 66,000,

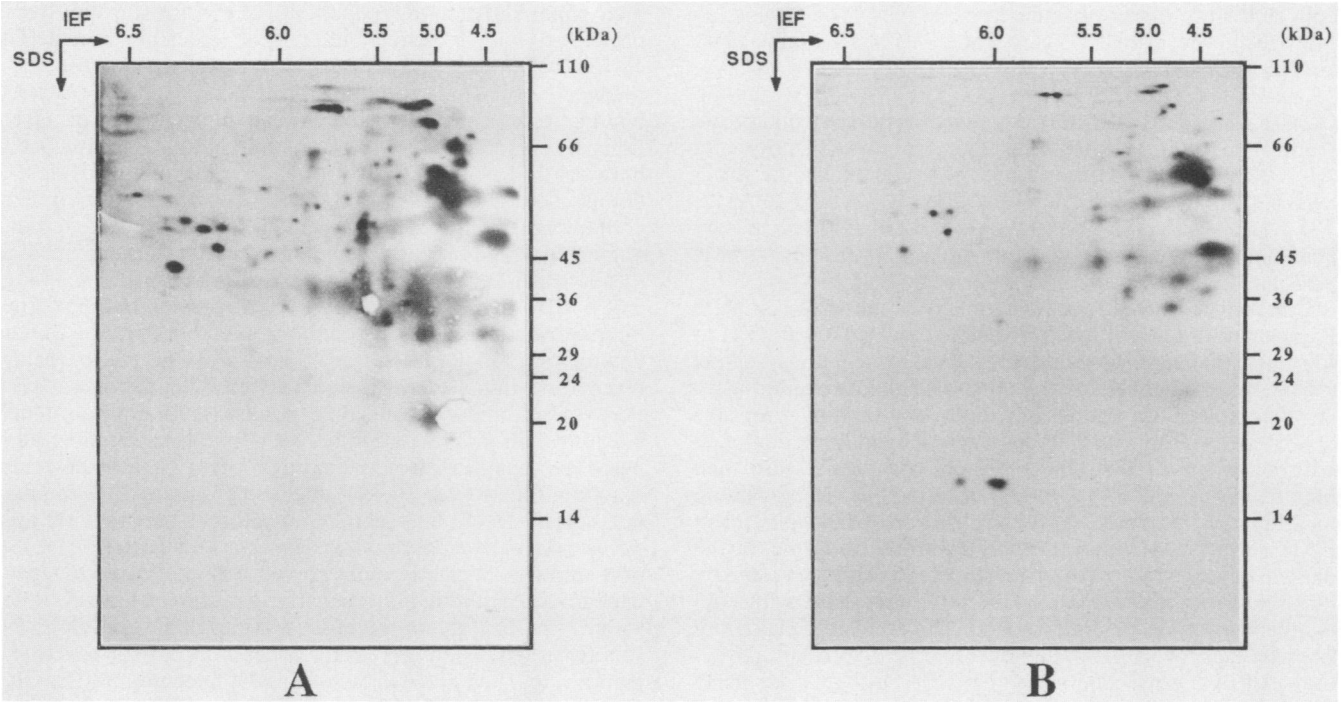


FIG. 1. Con A-binding glycoprotein patterns of *Agrocybe aegerita* homokaryons F31 (A) and F3 (B). Proteins were separated by two-dimensional SDS-PAGE and then transferred to a nitrocellulose filter. After transfer, the blot was treated with Con A and peroxidase, and the glycoprotein-Con A-peroxidase complexes were then colored with 4-chloro-1-naphthol as described in Materials and Methods. IEF, isoelectric focusing.

etc.). Standard protein and glycoprotein maps were compared by considering only qualitative differences (i.e., the presence or absence of spots).

RESULTS

Comparison of Con A-binding protein patterns of homokaryotic siblings. In order to measure the variation in the glycoproteins from homokaryotic strains, the total mycelial proteins of 11 postmeiotic homokaryotic siblings from a single wild-type dikaryotic strain were separated by two-dimensional SDS-PAGE and transferred to nitrocellulose filters, which were subsequently treated with Con A and peroxidase as described in Materials and Methods. Figure 1 shows an example of a two-dimensional separation of the glycoproteins synthesized in two homokaryons. The glycoproteins detected had apparent molecular weights ranging from 12,000 to 100,000 and isoelectric points ranging from 4.3 to 7.0. Standard glycoprotein maps of the 11 homokaryons were drawn (Fig. 2), and 253 glycoproteins were recorded for all of the homokaryotic strains. A comparison of the standard maps showed that there was great variability in the number of spots detected per homokaryon (57 spots for homokaryon F3, 127 spots for homokaryon F34). Only 18 spots were common to all of the homokaryons (Fig. 2), representing 14 to 26% of the spots for the strains.

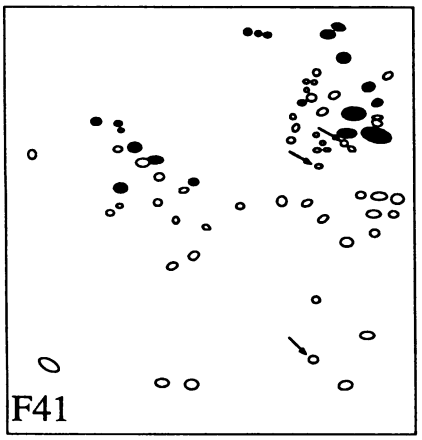
There was great diversity in the glycoprotein patterns, and we detected glycoproteins that were specifically synthesized by each of the homokaryons (Fig. 2, arrows). The numbers of glycoproteins that were specific to individual homokaryons were as follows: F3, 1; F41, 3; F31, 3; F24, 4; F12, 4; F9, 4; F30, 6; F19, 10; F25, 20; F6, 21; and F34, 22.

Comparison of two-dimensional patterns for glycoproteins from homokaryotic strains and the dikaryotic strains produced by anastomosis of these strains. The two-dimensional patterns for glycoproteins (Con A-binding proteins) from compatible homokaryons F30 (*A1B1*), F31 (*A2B2*), A17 (*A9B9*), and A117 (*A10B5*) were compared with the glycoprotein patterns for six dikaryons resulting from anastomosis of these homokaryons (F30/F31, F30/A17, F30/A117, F31/A17, F31/A117, and A17/A117).

The comparison of the standard maps of the six dikaryotic strains showed that spots which were common to all of the dikaryons represented 40 to 60% of the spots depending on the strain genotypes. Accordingly, glycoprotein variability appeared to be lower in dikaryotic strains than in homokaryons (14 to 26% common spots).

For a comparative analysis of the standard maps of the dikaryons with the maps of the homokaryons from which the dikaryons were derived (Fig. 3), the qualitative variations among glycoproteins were classified into six categories (Table 1). Our results showed that some of the glycoproteins synthesized by a homokaryon were not present in the dikaryon (for example, 19 spots specific to homokaryon F30

FIG. 2. Standard glycoprotein maps for 11 homokaryotic siblings. The glycoproteins that were common to all of the strains are indicated by solid spots; the glycoproteins that varied in their presence among the strains are indicated by open spots. The strain-specific glycoproteins are indicated with arrows. Standard maps were established by using three independent protein samples prepared from each strain; for each strain, nonreproducible spots were discarded.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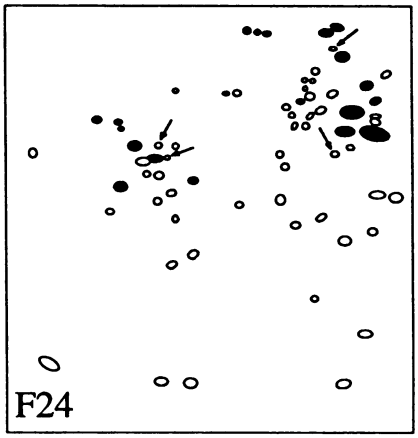
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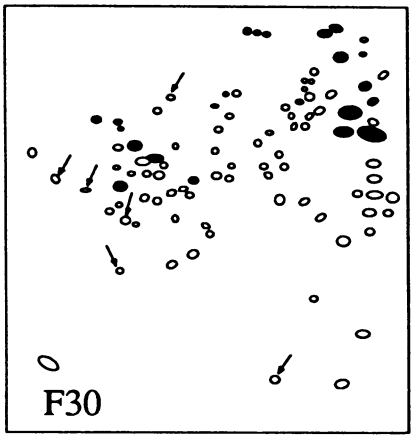
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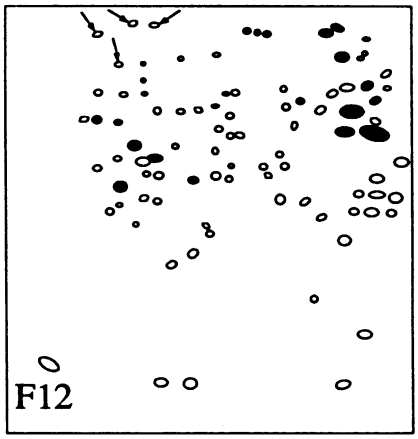
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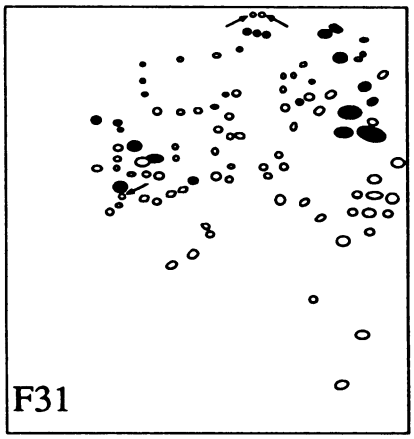
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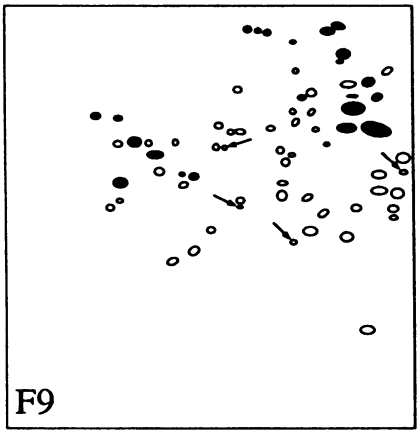
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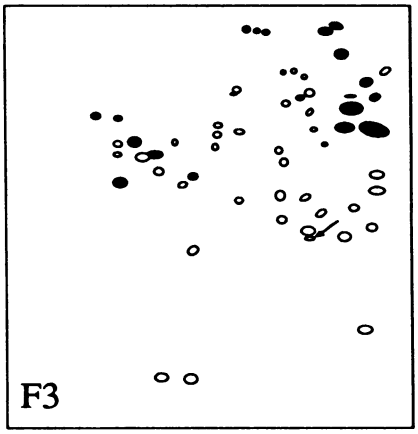
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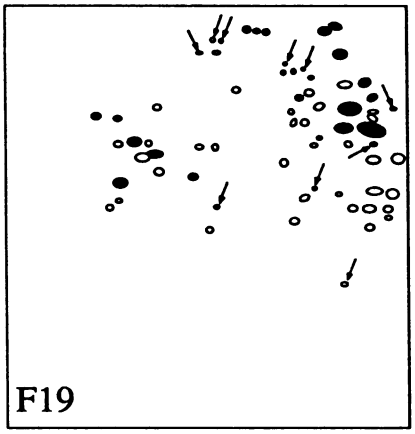
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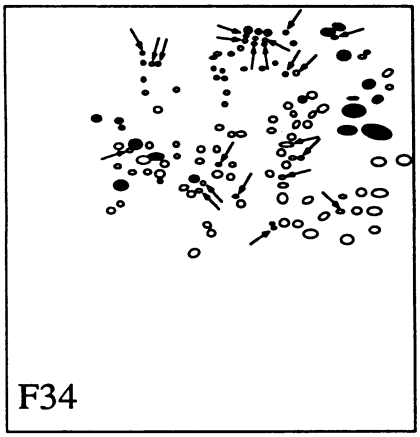
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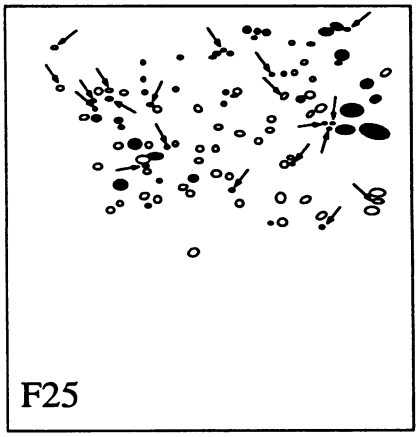
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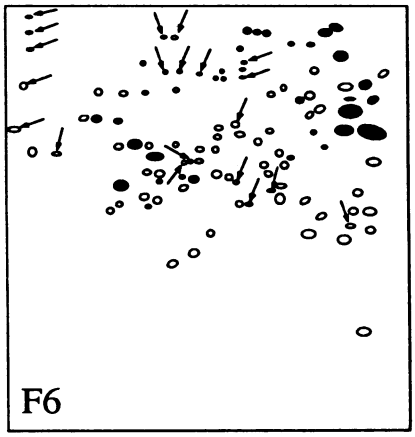
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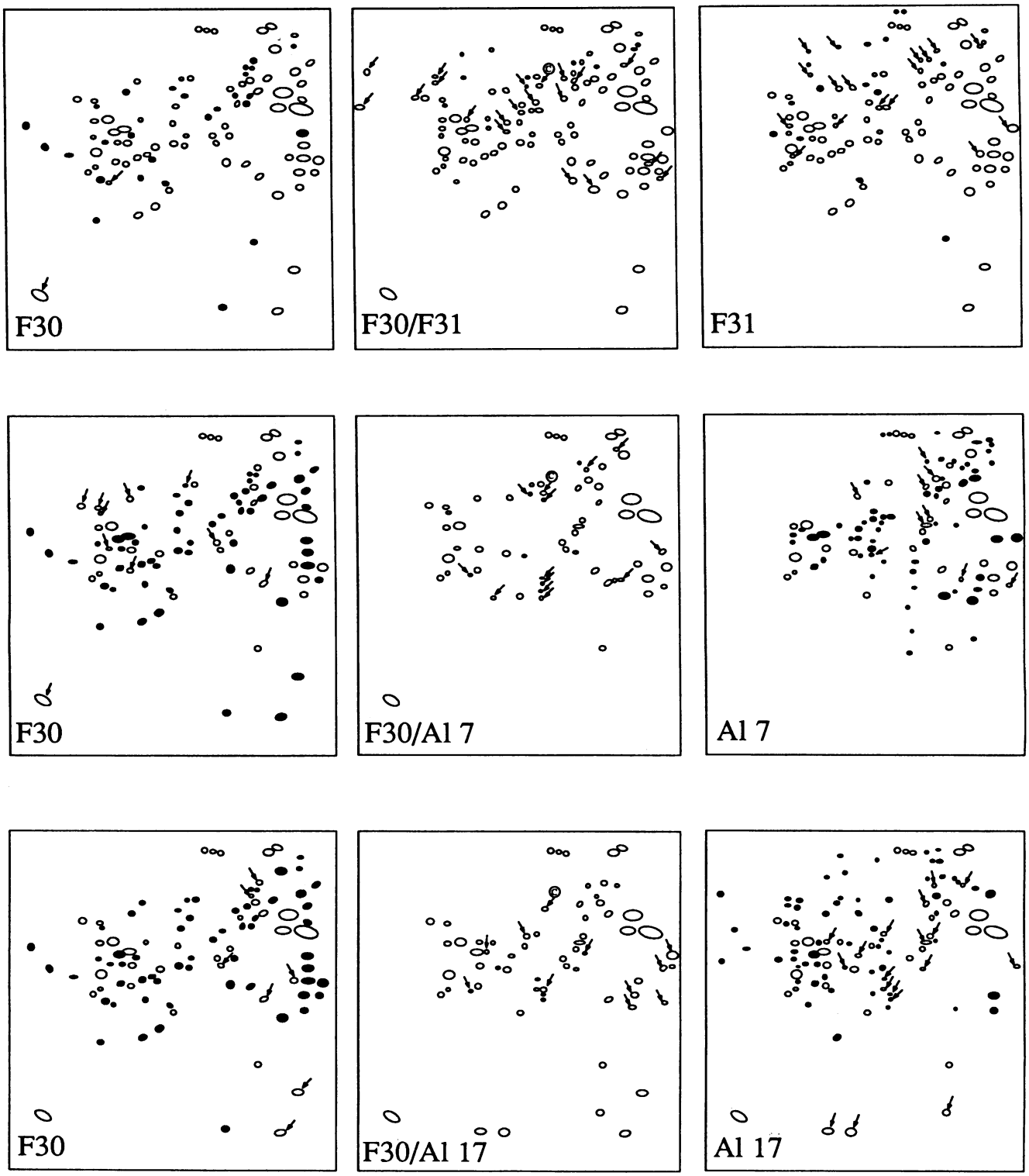

FIG. 3. Standard glycoprotein maps for four compatible homokaryons (F30, F31, A17, and A117) and for six dikaryons resulting from anastomosis of these homokaryons (F30/F31, F30/A17, F30/A117, F31/A17, F31/A117, and A17/A117). On the homokaryon maps, the glycoproteins which were absent in the dikaryons are indicated by solid spots (see Table 1); the glycoproteins that were specific to one homokaryon (were not found in the other homokaryon) and were present in the dikaryon are indicated with arrows. On the dikaryon maps, the glycoproteins that were specific to a dikaryon (were not found in the two homokaryons from which the dikaryon was derived) are indicated with arrows; among these, the glycoprotein which was common to all of the dikaryons is indicated by ©. Standard maps were established by using three independent protein samples prepared from each strain; for each strain, nonreproducible spots were discarded.

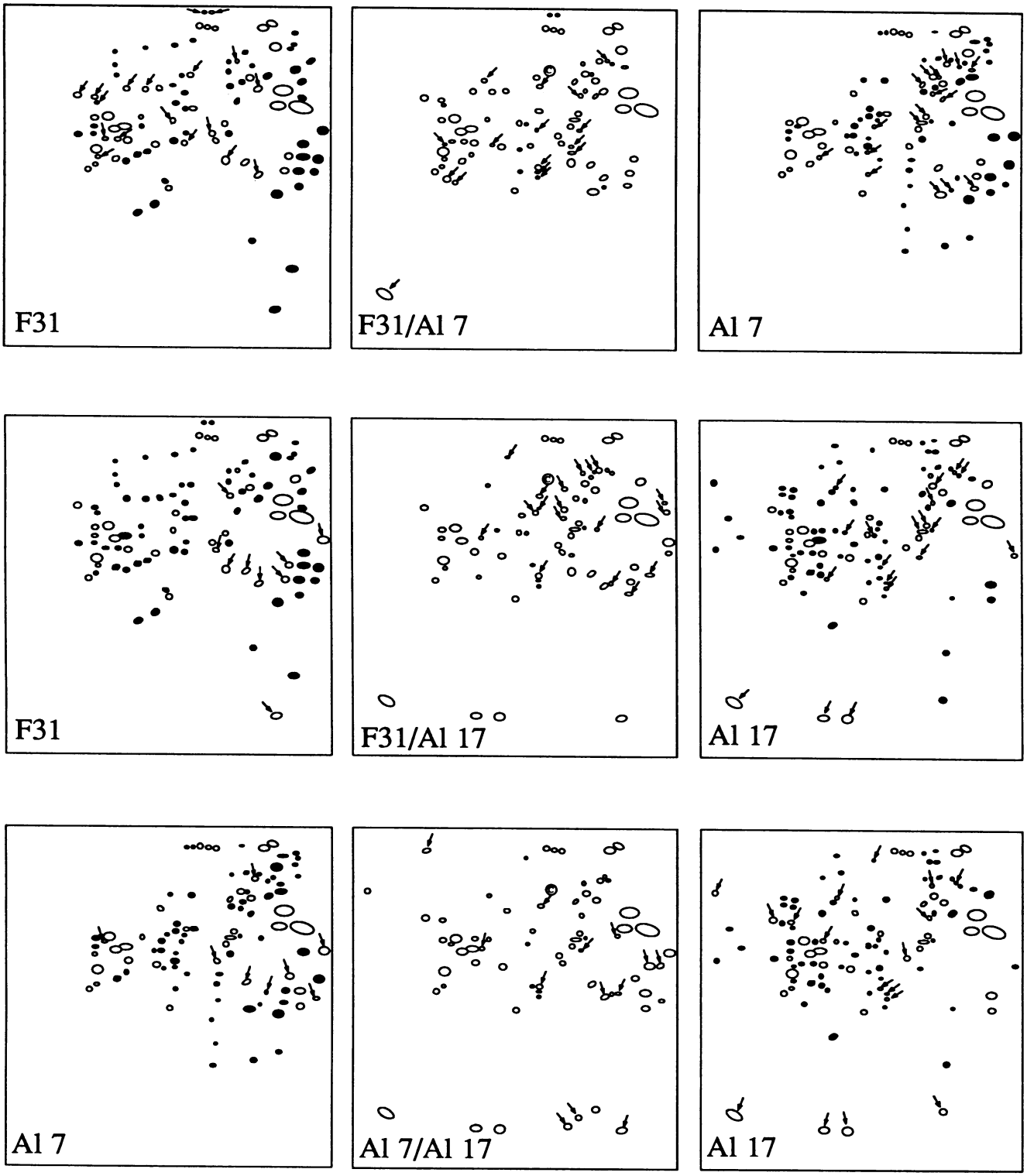

FIG. 3—*Continued.*

were missing in dikaryon F30/F31). The numbers of glycoproteins expressed in homokaryons which were missing in dikaryons depended on the homokaryons involved (19 spots of F30 and 6 spots of F31 were not detected in dikaryon F30/F31) and also on the associations (40 spots of F30 were missing in dikaryon F30/A17, but only 19 spots were absent in dikaryon F30/F31). In dikaryons, missing glycoproteins could represent 45% of the spots of a homokaryon (for example, F30 and A17 homokaryotic components in dikaryons F30/A117 and F30/A17, respectively).

TABLE 1. Qualitative variations in glycoproteins in six dikaryotic strains obtained by pairing four homokaryotic strains

Dikaryon (homokaryon 1/homokaryon 2)	Glycoproteins of the haploid nuclei						Variations specific to dikaryons		Relationship between haploid nuclei[c]
	No. of spots specific to homokaryon 1 and present in dikaryon[a]	No. of spots specific to homokaryon 1 and absent in dikaryon	Expression level of homokaryon 1[b]	No. of spots specific to homokaryon 2 and present in dikaryon	No. of spots specific to homokaryon 2 and absent in dikaryon	Expression level of homokaryon 2[b]	No. of spots present in dikaryon and absent in homokaryons 1 and 2	No. of spots present in homokaryons 1 and 2 and absent in dikaryon	
F30/F31	+2	−19	−17	+17	−6	+11	+20	−6	F30 < F31
F30/A17	+10	−40	−30	+9	−44	−35	+13	−16	F30 > A17
F30/A117	+7	−43	−36	+18	−43	−25	+11	−16	F30 < A117
F31/A17	+18	−31	−13	+17	−33	−16	+16	−19	F31 > A17
F31/A117	+9	−39	−30	+19	−38	−19	+18	−23	F31 < A117
A17/A117	+8	−39	−31	+17	−37	−20	+13	−19	A17 < A117

[a] Spots specific to a homokaryon were present in that homokaryon and absent in the other homokaryon of a dikaryon.
[b] Values were obtained by adding the values for the number of spots specific to the homokaryon and present in the dikaryon and the number of spots specific to the homokaryon and absent in the dikaryon.
[c] Relationships between homokaryotic components were determined by comparing their expression levels.

In order to measure the expression levels of homokaryotic components in dikaryons, we determined the numbers of homokaryon-specific glycoproteins which were expressed in dikaryons (positive values) and were absent in dikaryons (negative values); the resulting values (Table 1) represented the expression levels of the haploid nuclei. This led us to compare the expression levels of both homokaryotic components in dikaryons and to estimate the interrelationships that existed between them. For example, the level of protein expression in the F30 component was less than the levels of expression of the F31 and A117 components in dikaryons F30/F31 and F30/A117, respectively, and was more than the level of expression of the A17 component in dikaryon F30/A17. These results were symbolized as follows: F30 < F31, F30 < A117, and F30 > A17 (Table 1). On the basis of the different interrelationships evaluated, the classification of homokaryotic components with regard to their glycoprotein expression levels was as follows: A117 (*A5B10*) > F31 (*A2B2*) > F30 (*A1B1*) > A17 (*A9B9*).

Identification of a glycoprotein specific to the dikaryotic state. In dikaryons some new glycoproteins were synthesized and did not occur in the homokaryons from which the dikaryons were derived; for example, 20 new glycoproteins were detected in dikaryon F30/F31. A comparison of the glycoprotein patterns of mixed homokaryons (i.e., compatible mycelia mixed just prior to protein extraction) with the glycoprotein patterns of the dikaryons formed by anastomosis of these homokaryons confirmed that the new glycoproteins were present exclusively in the dikaryons. Some of these glycoproteins were specific to individual dikaryotic genotypes; 3, 6, 8, 9, 9, and 12 glycoproteins were specific to dikaryons F30/A117, F30/A17, A17/A117, F31/A17, F31/A117, and F30/F31, respectively.

Among the glycoproteins that were specifically expressed in dikaryons, one was detected in all of the dikaryons (indicated by © on the standard dikaryon maps in Fig. 3); since this glycoprotein was never found in homokaryons, it may be specific to the dikaryotic state. This glycoprotein was named gpAa-65; it had an apparent molecular weight of 65,000 and an isoelectric point of 5.8.

Protein pattern variations in compatible homokaryons and the dikaryons resulting from anastomosis of these homokaryons. The same type of comparative analysis described above for glycoproteins was performed with proteins separated by two-dimensional SDS-PAGE and visualized by silver nitrate staining. Figure 4 shows an example of a two-dimensional protein pattern. A comparison of the standard protein maps established for the dikaryotic strains showed that, depending on the strain, 93.3 to 95.4% of the proteins were common to all of the dikaryons.

The protein expression levels for the homokaryotic components were estimated for each dikaryon and were compared as described above for glycoproteins (Table 2). The proteins exhibited low levels of variability compared with glycoproteins; the proportion of homokaryotic component proteins that were expressed or absent in the dikaryons was never more than 3.7% of the spots, compared with a value of 45% for glycoproteins. We classified the homokaryotic components according to their protein expression levels as follows: A117 (*A5B10*) = F31 (*A2B2*) > F30 (*A1B1*) > A17 (*A9B9*).

In the dikaryons, the numbers of proteins which were newly expressed (Table 2) were low. Only one protein was specific to dikaryon F30/A117, one protein was specific to F31/A17, one protein was specific to F31/A117, three proteins were specific to F30/F31, three proteins were specific to A17/A117, and four proteins were specific to F30/A17. No dikaryon-specific protein common to all of the dikaryotic strains was detected.

DISCUSSION

All of the mycelia analyzed in this study were cultivated under standard conditions (identical growth conditions, growth rates, and times of harvest). Under these conditions, nearly identical protein patterns (25) or glycoprotein patterns

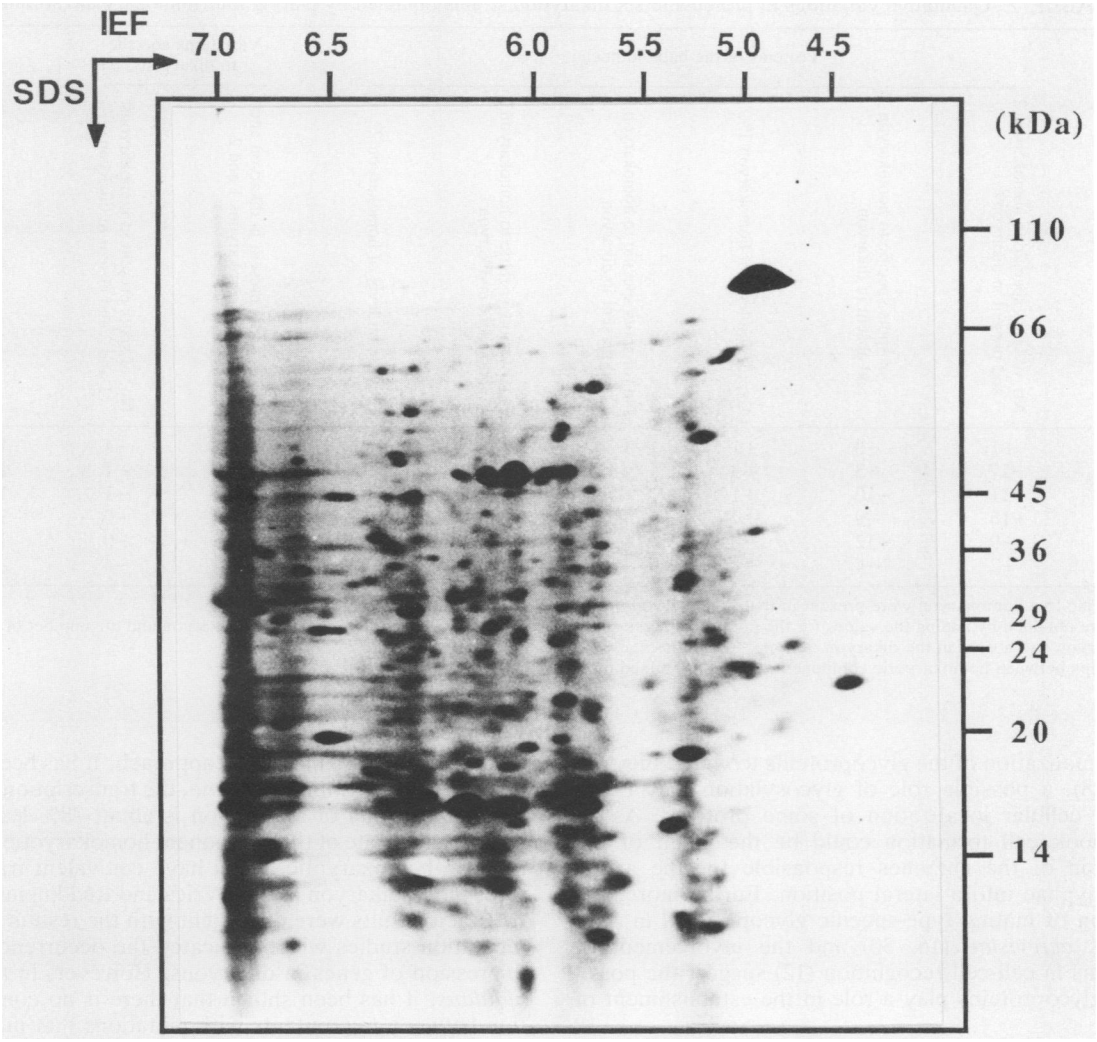

FIG. 4. Two-dimensional gel electrophoresis of total proteins from *Agrocybe aegerita* homokaryon F30. Protein sample preparation and separation were as described in Materials and Methods. The proteins were detected by silver staining. IEF, isoelectric focusing.

were obtained for independent cultures of each mycelium. Furthermore, standard protein and glycoprotein maps for each strain were constructed by using three independent protein extracts in order to include only the reproducible spots. The repeatability of the results allowed us to perform comparative studies.

Our comparison of the standard glycoprotein maps for homokaryons and dikaryons showed that there was great variability among the glycoproteins compared with the variability among the proteins previously studied in *Agrocybe aegerita* (25), and our maps permitted identification of glycoproteins specifically synthesized by each strain. Accordingly, glycoprotein patterns appear to be useful for differentiating closely related strains, such as homokaryotic siblings and dikaryons resulting from anastomosis of these homokaryons. Two-dimensional glycoprotein pattern analysis may be used for identifying commercially valuable strains and to ensure patent protection of these strains. For this purpose, analysis of glycoproteins might be complementary to isoenzymatic studies (24).

We previously determined that 97% of the total proteins were common to the 11 homokaryons included in this study (25). In comparison, the glycoproteins varied greatly since glycoproteins which were common to all of the strains represented only 16 to 24% of the glycoproteins in the strains. A similar difference was observed in dikaryotic strains; common spots accounted for 93.3 to 95.4% of the proteins, whereas they represented 40 to 60% of the glycoproteins, depending on the strain. Despite the fundamental interest of glycoproteins, they have not been studied in filamentous fungi. Nevertheless, developmental studies with the myxomycete *Dictyostelium discoideum* have shown that there are only a few changes in the total protein profiles during development, while many glycoproteins are differentially regulated (3). The fact that the glycoproteins of *Agrocybe aegerita* are subjected to considerable qualitative variations compared with proteins and the fact that few qualitative differences have been found between homokaryotic and dikaryotic mycelia in *Schizophyllum commune* (7, 22) with regard to polysomal RNA content, strongly suggest that glycosylation plays a major role in the control of dikaryosis. Since the structure of glycan moieties directs the

TABLE 2. Qualitative variations in proteins in six dikaryotic strains obtained by pairing four homokaryotic strains

Dikaryon (homokaryon 1/homokaryon 2)	Proteins of the haploid nuclei						Variations specific to dikaryons		
	No. of spots specific to homokaryon 1 and present in dikaryon[a]	No. of spots specific to homokaryon 1 and absent in dikaryon	Expression level of homokaryon 1[b]	No. of spots specific to homokaryon 2 and present in dikaryon	No. of spots specific to homokaryon 2 and absent in dikaryon	Expression level of homokaryon 2[b]	No. of spots present in dikaryon and absent in homokaryons 1 and 2	No. of spots present in homokaryons 1 and 2 and absent in dikaryon	Relationship between haploid nuclei[c]
F30/F31	+1	−0	+1	+2	−0	+2	+4	−4	F30 < F31
F30/A17	+17	−8	+9	+3	−4	−1	+5	−1	F30 > A17
F30/A117	+11	−10	+1	+7	−1	+6	+3	−1	F30 < A117
F31/A17	+16	−9	+7	+2	−4	−2	+6	−0	F31 < A17
F31/A117	+9	−12	−3	+2	−5	−3	+4	−0	F31 = A117
A17/A117	+2	−4	−2	+6	−5	+1	+12	−4	A17 < A117

[a] Spots specific to a homokaryon were present in that homokaryon and absent in the other homokaryon of a dikaryon.
[b] Values were obtained by adding the values for the number of spots specific to the homokaryon and present in the dikaryon and the number of spots specific to the homokaryon and absent in the dikaryon.
[c] Relationships between homokaryotic components were determined by comparing their expression levels.

compartmentalization of the glycoproteins to which they are attached (28), a possible role of glycosylation may be to change the cellular localization of some proteins. As an example, hook cell formation could be the result of the delocalization of the enzymes responsible for the apical growth of hyphae into a lateral position. Furthermore, the identification of mating type-specific glycoproteins in *Saccharomyces cerevisiae* (16, 30) and the involvement of glycoproteins in cell-cell recognition (12) suggest the possibility that glycoproteins play a role in the establishment of dikaryons.

Our comparison of protein and glycoprotein expression in the haploid nuclei in the homokaryotic state and in various paired combinations in the dikaryotic state showed that in dikaryons the two haploid nuclei are differentially regulated. The expression level of each nucleus varies according to its genotype and according to the complementary nuclear genotype. A compilation of the relationships observed among the four haploid nuclei according to their associations led to establishment of classifications of the homokaryotic components with respect to (i) their protein expression levels (A117 [*A5B10*] = F31 [*A2B2*] > F30 [*A1B1*] > A17 [*A9B9*]) and (ii) their glycoprotein expression levels (A117 [*A5B10*] > F31 [*A2B2*] > F30 [*A1B1*] > A17 [*A9B9*]). Except for the divergence in interrelationships observed with A117 and F31, which was probably due to the greater variation in glycoproteins than in proteins, the comparisons of both the protein and glycoprotein patterns led to classification of the homokaryotic components in virtually the same order. This result suggests that protein synthesis and glycosylation are similarly regulated in dikaryons. At the present time, it is difficult to speculate whether haploid nuclear expression is controlled by incompatibility genes or by other genes. Dissymmetric regulation in the mitotic division of both nuclei has been reported in the basidiomycete *Schizophyllum commune* (20), but this was the first time that dissymmetry in the expression of both nuclei in a basidiomycete was directly demonstrated by a molecular approach. It has been reported that in *Schizophyllum commune*, the transcription rate of the individual nuclei of a dikaryon is about 28% less than the transcription rate of the component homokaryotic nuclei and that the homokaryotic nuclei have equivalent transcription rates in the dikaryon (6). De Vries and Reddingius (6) stated that their results were consistent with the results of complementation studies which indicated the occurrence of equal expression of genes in dikaryons. However, in *Aspergillus nidulans*, it has been shown that there is no complementation between *fac* mutants with mutations that map at three different loci when they are paired in the three possible combinations in heterokaryons, whereas complementation does occur in these mutants in the diploids (1). In any case, our results show that there is dissymmetric expression of proteins and glycoproteins in the two component nuclei of dikaryons. The fact that some haploid nuclei have higher expression levels than others could be exploited in breeding programs, notably by using homokaryotic components in which a maximal number of genes are expressed in the dikaryotic state when maximal expression is desirable.

In the dikaryon glycoprotein study, we identified a glycoprotein (gpAa-65) which was always detected in all of the dikaryons. This Con A-binding glycoprotein, which was never detected in the 13 homokaryons analyzed, appears to be specific to the dikaryotic state. This suggests that dikaryotic strains can be recognized by a simple analysis (two-dimensional analysis of glycoproteins) without the need for a time-consuming fruiting test. In *Agrocybe aegerita*, dikaryons are easily identifiable by microscopy, but in some basidiomycetes dikaryon morphology is indistinguishable from homokaryon morphology. The detection of gpAa-65 (or a related protein) in such mushrooms might be very useful for breeding, particularly in *Agaricus bisporus*. In the pseudohomothallic species, genetic improvement requires the screening of uninucleated spores and the recognition of heterokaryotic mycelium after protoplast regeneration (4). It

should be noted that the time required for identification of heterokaryotic strains could be greatly shortened by using a specific antiserum directed against a specific glycoprotein, such as gpAa-65 of *Agrocybe aegerita*, as described previously for immunodetection of *Agaricus bisporus* tyrosinase (15).

Although only one Con A-binding protein was found to be specific to the dikaryotic state in *Agrocybe aegerita*, the study of glycoproteins seems to be more relevant than the study of proteins for identifying gene products that are implicated in specific structures and functions of dikaryons. Indeed, no silver-stained protein was found to be common to all dikaryons. By analogy to the basidiomycete *Schizophyllum commune*, which is capable of coding for about 10,000 different proteins (5), the 500 silver-stained proteins resolved by two-dimensional electrophoresis in *Agrocybe aegerita* should represent 5% of the synthesizable proteins. Accordingly, it is possible that proteins specific to dikaryons could not be resolved on our two-dimensional gels or may be present in quantities too low to be detected. This is the case for glycoprotein gpAa-65 since it was detected with the Con A-peroxidase method but not by silver staining. In this study, we focused on Con A-binding proteins; it is possible that comparable studies in which other lectins are used may lead to the identification of additional glycoproteins that are implicated in the dikaryotic process.

Our data clearly show that when two homokaryotic components are associated in a dikaryon, the levels of expression of these homokaryotic components are asymmetric. These results open the way to an understanding of the control mechanisms that regulate the expression of the two haploid nuclei in dikaryons. Moreover, the study of glycoprotein gpAa-65, which was found to be specific to the dikaryotic state, could contribute to a better understanding of the dikaryotic process. The purification of this glycoprotein by using Con A (26) may be the first step in elucidating its function.

ACKNOWLEDGMENTS

We thank Joanne Marie and Kathrin Candresse for reading the manuscript.

This work was supported by grants from the Conseil Scientifique de l'Université de Bordeaux II, the Institut National de la Recherche Agronomique, and the Lur Berri Food Co.

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