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Genetics

Mutants of *Neurospora crassa* that alter gene expression and conidia development

(trans-acting mutants/gene regulation/defective development/spore formation)

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ABSTRACT **Several genes have been identified that are highly expressed during conidiation. Inactivation of these genes has no observable phenotypic effect. Transcripts of two such genes, *con-6* and *con-10*, are normally absent from vegetative mycelia. To identify regulatory genes that affect *con-6* and/or *con-10* expression, strains were prepared in which the regulatory regions for these genes were fused to a gene conferring hygromycin resistance. Mutants were then selected that were resistant to the drug during mycelial growth. Mutations in several of the isolates had trans effects; they activated transcription of the corresponding intact gene and, in most isolates, one or more of the other *con* genes. Most interestingly, resistant mutants were obtained that were defective at different stages of conidiation. One mutant conidiated under conditions that do not permit conidiation in wild type.**

When *Neurospora crassa* is grown in a liquid medium, it forms a meshwork of branched, fused, vegetative hyphae, called a mycelium. Mycelia are induced to sporulate simply by air exposure. This treatment induces a series of morphological changes that culminate in the production of multinucleate asexual spores, called macroconidia, or conidia (1). In synchronized cultures undergoing conidiation, the process is completed in 12–14 hr (2). Initially, upon induction, aerial hyphae are produced. These undergo apical budding, forming what are called minor constriction chains. Continued apical growth of these chains produces major constriction chains; these have pronounced constrictions between adjacent cells (1). Nuclei then migrate into the proconidial chains, and cross-walls are laid down between adjacent proconidia. Finally, each of these cells matures to become a free conidium.

Analyses of the protein and mRNA species present in mycelia, aerial hyphae, and conidia have revealed significant differences in gene expression (3–5). A differential screening procedure was used to clone genes that are preferentially expressed during conidiation (4). Four of these genes, *con-6*, *con-8*, *con-10*, and *con-13*, have been sequenced, and their expression has been examined (6–10). A fifth conidiation-specific gene, *eas*, also has been characterized (11, 12); *eas* encodes the rodlet protein that coats the surface of the mature conidium.

con-6 and *con-10* encode 93- and 86-residue polypeptides, respectively; the functions of these polypeptides are unknown. con-6 and con-10 transcripts and proteins are normally undetectable in vegetative mycelium but appear at high levels during conidiation and in mature conidia; these transcripts and proteins disappear a few hours after conidia germinate (6, 13). The expression pattern of these genes closely parallels that of *eas* (11, 12). The con-6 and con-10 proteins are present in a second type of asexual spore, the microconidium (10, 13), and the con-10 protein is present in sexual spores (10, 13). Expression of *con-6* and *con-10* can also be induced in vegetative mycelial cultures by light exposure or by imposing conditions that induce circadian rhythm (10, 13–15). We describe here the selection and partial characterization of mutants that express *con-6* and *con-10* aberrantly during vegetative mycelial growth. Some of these mutants were found to be blocked in conidiation, whereas others exhibited different developmental abnormalities.

MATERIALS AND METHODS

Strains and Plasmids. Strain CH10 was constructed by homologous integration, using pCH10, a plasmid containing a *con-10* translational fusion to the hygromycin phosphotransferase gene (*hph*) (16). Integration was at the *his-3* locus of Fungal Genetics Stock Center (FGSC) strain 462: *his-3 A*. The integrated plasmid consisted of pBluescript KS+ (Promega) vector containing *con-10* DNA from positions 1139 to 1935 (8); this segment contains the first 40 codons of *con-10*. The *con-10* DNA is fused in-frame to the second codon of *hph* followed by the *Aspergillus nidulans trpC* transcription termination region from plasmid pDH25 (16). The plasmid also contains a 5′ truncated copy of the *his-3* gene that allows reconstitution of an intact copy of the *his-3* gene by homologous integration of the circular plasmid at *his-3*. Integration results in duplicate *his-3* sequences flanking plasmid sequences and the *con-10–hph* fusion.

Strain CH6 contains a single copy of a *con-6* translational fusion to *lacZ* of *Escherichia coli* (pBW701) integrated at *his-3* of strain his-3 a (FGSC no. 6524) and two ectopic copies of a *con-6* translational fusion to *hph*. *con-6* DNA (nt 1–1212) (6) was fused to the *hph/trpC* terminator region described above and transferred to pBT3, a plasmid containing a mutant β-tubulin gene that confers benomyl resistance (17). This plasmid, pBW720, was used to transform strain 6524(pBW701)-3, a strain containing the *con-6–lacZ* fusion at the *his-3* locus (6), selecting for benomyl resistance. Southern blot analysis was used to examine the structure of the regions containing integrated plasmid DNA.

Mutagenesis and Selection Conditions. Cells of CH6 and CH10 embedded in agar are resistant to low levels of hygromycin (0.2 mg/ml) but are sensitive to hygromycin at 0.5–0.8 mg/ml. Mutagenesis was performed by using UV light (18). Approximately 32% of CH6 and 15% of CH10 conidia were killed by the UV treatment. Samples containing 1–5 × 10^6 irradiated conidia were suspended in 15 ml of Vogel's minimal agar plus sorbose (18, 19) plus hygromycin at 1.5 mg/ml and poured into a 100-mm Petri dish. After the agar in each plate solidified, 20 ml of the same hygromycin agar was added

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as a top layer. Colonies that reached the surface of the top agar 4–7 days after plating were picked for analysis.

Culture Conditions. Mycelial cultures were grown in Vogel's minimal medium/1.5% sucrose. Fifty milliliters of medium was autoclaved in dichlorodimethylsilane-treated 125-ml flasks, and Tween 80 was added to a final 0.1% concentration. Flasks were inoculated with either conidia or mycelial fragments, and cultures were grown for 1–2 days on a rotary shaker at 200 rpm at 34°C with constant illumination. Mycelia were harvested and shredded in a Waring blender by two 15-sec pulses. Mycelial fragments were used to inoculate a second flask containing the same medium. This flask was grown as above for 16–20 hr before harvesting. Mycelial growth rate was measured by a modification of the race tube procedure (18).

RNA and Protein Extraction. RNA was isolated from mycelia or conidia by disrupting suspensions with 0.5-mm glass beads in a mini-beadbeater (Biospec Products, Bartlesville, OK) in the presence of phenol/chloroform and SDS-containing buffer, as described (20), except that 10 mM EDTA was added to the extraction buffer. Radiolabeled RNA probes complementary to con-6, con-8, con-10, and eas mRNAs were prepared by *in vitro* transcription from linearized subclones prepared in this laboratory—pCON6-6, pRB2, pBW100, and pEAS, respectively—using either T7 or T3 RNA polymerases. A radiolabeled β-tubulin DNA probe was prepared by random-primed synthesis of linearized pBT3. Probes were used in RNA blot analyses (20) at a hybridization temperature of 60°C. Protein extracts were prepared by breaking cells in assay buffer (21) with 0.5-mm glass beads in 2-ml screw-capped tubes in the mini-beadbeater for 1 min. β-Galactosidase was assayed as described (22); the values presented are the averages for two cultures, each assayed in duplicate.

Morphological Studies. Aerial cultures of CH10 and CH10 derivatives were grown on drops of solidified medium on top of coverslips. Coverslips were inverted onto water on slides and viewed with an Olympus (New Hyde Park, NY) BH2-RFC microscope using a ×40 objective and photographed using Ektachrome 100 HC film. Conidia of strain CH10-7 were inoculated into minimal medium and grown for 20 hr at 34°C before photography.

Genetic Analyses. CH10 mutants were crossed to ORS-6a to test for cosegregation of morphological defects with aberrant *con-10* expression. Because duplicated *his-3* sequences flank the *con-10–hph* gene fusion, such crosses have several potential outcomes. The duplicated segments of *his-3* could undergo recombination, leading to loss of the gene fusion. Further, depending on location of the crossover, a His^+ or His^- recombinant could be produced. In addition, the duplicated *his-3* segments could be mutated by repeat induced point mutations (23), resulting in a His^- phenotype.

Progeny from crosses with CH10-1 (Table 1) were screened for resistance to low levels of hygromycin to identify morphologically mutant and wild-type progeny containing the *con-10–hph* fusion gene. These progeny were then tested for growth while embedded in agar medium containing hygromycin at 1.5 mg/ml to assess high-level expression of the *con-10–hph* fusion. Because we found intermediate levels of drug resistance among the progeny with this assay, the more direct approach of Northern analysis was used to examine *con-10* expression in progeny of the CH10-2, CH10-3, CH10-4, and CH10-5 backcrosses. RNA was prepared from mycelial cultures of progeny from the crosses of these strains to ORS-6a. No progeny from crosses of strain CH10-6 to ORS-6a had the CH10-6 phenotype; no further genetic analysis was done. The progeny of crosses with strain CH10-7 were not tested because CH10-7 produces conidia during growth in liquid medium. Strain CH10-8 was not examined because it displayed wild-type morphology.

RESULTS

Selection of Mutants with Altered Expression of *con-10*. Strain CH10 contains a single copy of the *con-10–hph* fusion integrated at the *his-3* locus. This *con-10* fusion construct contains 517 bp upstream of the transcription start site. Growth of CH10 in agar is inhibited by hygromycin at 1500 μg/ml. Resistant mutants were selected after treatment of conidia with UV light. After 4–7 days at 34°C ≈20–50 resistant colonies were visible per plate. Sixty resistant colonies were selected for analysis. Because these might have been heterokaryons, each isolate was purified by three successive rounds of plating of conidia or mycelial fragments on hygromycin agar. During the initial rounds of purification, it was evident that several had abnormal morphologies. These isolates were considered representative of the colony from which they were derived. Selected isolates were screened for trans effects using Northern blot analyses. Eight isolates had elevated levels of the con-10 transcript (data not shown); five of these produced mature conidia, and three did not. The conidiating strains were designated CH10-2, CH10-5, CH10-6, CH10-7, and CH10-8, and the conidiation-defective strains were designated CH10-1, CH10-3, and CH10-4.

Characterization of Resistant Isolates from CH10. The growth rate of each selected CH10 isolate, except CH10-8, was slower than that of CH10 (Table 1). CH10-3 and CH10-4 were mostly aconidial; however, after extended incubation periods, proconidial chains were seen. These chains produced few free conidia (Fig. 1). CH10-1 is conidia-separation defective; it produced proconidial chains, but few free conidia. Strains CH10-2, CH10-5, CH10-6, CH10-7, and CH10-8 were indistinguishable from wild type during conidiation. CH10-4 cultures often displayed "bumpy" aerial hyphae indicative of some morphological defect in the formation of proconidial chains; CH10-4 mycelia were similar in appearance to wild type during growth in liquid medium. CH10-6 mycelium was unusually dense when grown on agar. In addition, CH10-6 conidia were readily dispersed during handling of race tubes; this is atypical of wild type, parent CH10, or the other CH10 mutants examined. Microscopic examination revealed that many CH10-6 conidia were elongated (Fig. 1). An increase in the number of arthroconidia-like conidia formed from minor constriction chains or from aerial hyphae might account for the large number of elon-

Table 1. Properties of mutants derived from CH10

	Test or assay					
	mRNA†				Growth	con
Strain*	con-8	con-6	con-10	eas	rate‡	type§
CH10 (C)	+	+	+	+	5.0	WT
CH10 (M)	−	−	−	−	5.0	WT
CH10-1	+	±	+	+	2.3	CSP
CH10-2	+	±	±	+	2.7	WT
CH10-3	−	−	+	+	1.0	Acon
CH10-4	−	−	+	+	1.2	Acon
CH10-5	+	−	±	−	1.9	WT
CH10-6	+	−	+	−	0.9	Ec
CH10-7	+	+	+	+	3.7	Hcon
CH10-8	+	±	±	−	4.4	WT

*Conidia of CH10 [CH10 (C)] were assayed for mRNA; mycelia of CH10 [CH10 (M)] and the mutants were also assayed.
†mRNA was (+) or was not (−) detected in Northern blots; ± indicates that mRNA was detectable at low levels, or detection varied with different RNA preparations.
‡Growth rate of mycelia in mm/hr.
§Conidiation phenotype: WT, mature conidia; Ec, elongated conidia resembling arthroconidia; Hcon, hyperconidiation; Acon, aconidial; CSP, conidia separation defective.

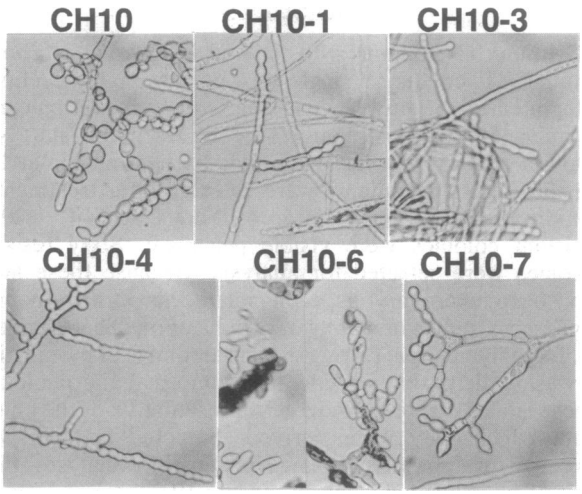

FIG. 1. Phenotypes of mutants derived from strain CH10. Hyphae from aerial cultures of mutants except CH10-7 were placed in water on a slide and viewed by bright-field illumination. The CH10 field illustrates wild-type conidiation, which was also seen in CH10-2, CH10-5, CH10-7, and CH10-8. CH10-1 has a conidial separation-defective phenotype; it does form major constriction chains, but they rarely release free conidia. CH10-3 is aconidial; its hyphae do not develop constrictions. CH10-4 is aconidial and displays "bumpy" hyphae. CH10-6 frequently has elongated conidia. CH10-7 forms conidiophores in liquid culture; wild-type strains do not.

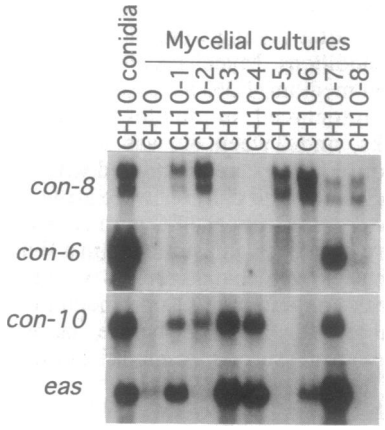

FIG. 2. Northern blots of RNA isolated from CH10 and its mutants. RNA was extracted from mature CH10 conidia and from mycelia formed in liquid cultures of CH10 and its mutants and was separated by electrophoresis and probed as described. Twenty micrograms of RNA was loaded in each lane. Results were obtained with ^{32}P-labeled probes containing con-8, con-6, con-10, or eas antisense RNA. Some RNA preparations did not contain elevated levels of con-10 mRNA. The explanation for this variability is unknown.

gated spores. When CH10-7 was grown on agar, it behaved much like wild type and conidiated normally. However, it was atypical in that it formed conidiophores when grown embedded in agar or in liquid minimal medium (Fig. 1). These conidiophores resembled wild-type aerial conidiophores. CH10-7 appears to have a "hyperconidiation" phenotype.

All CH10 mutants except CH10-5 were fertile as female parents. In repeated attempts to use CH10-5 as a female parent, we could not detect either protoperithecia or perithecia. Strain CH10-2 was remarkable in that it produced abundant protoperithecia throughout the agar of slants containing crossing medium. Submerged protoperithecia were seen >1 cm beneath the agar surface (data not shown); protoperithecia are typically formed only at or near the agar surface.

RNA samples extracted from mycelia of the CH10 mutants were analyzed for several *con* gene transcripts (Table 1). All mutants had elevated levels of intact con-10 mRNA produced from the intact endogenous copy of the gene (Fig. 2); however, there was some variation in the *con* gene mRNA content of samples prepared in different experiments. All CH10 mutants except CH10-3 and CH10-4 had elevated levels of the con-8 mRNA doublet (Fig. 2). con-6 mRNA was only detected in CH10-7. Because CH10-7 conidiates in liquid medium, the con mRNAs it produces probably reflect activation of development. Low levels of con-6 mRNA were detected in CH10-1, CH10-2, and CH10-8. CH10-1, CH10-2, CH10-3, CH10-4, and CH10-7 had increased levels of the eas transcript (Table 1, Fig. 2).

Genetic Analysis of CH10 Mutants. Genetic analyses were done to determine if the mutations conferring resistance to hygromycin also caused abnormal development. Crosses of strain CH10-1 by wild type gave equal numbers of mutant (altered morphology) and wild-type progeny. Both categories bearing the *con-10–hph* fusion (judged by growth on drug at 200 μg/ml) were assayed for ability to grow on hygromycin at 1500 μg/ml. None of the 10 morphologically wild-type progeny grew in the presence of this drug level. The morphological mutant progeny also were examined. However, because repeat induced point mutations (23) could have partially inactivated the *con-10* promoter segment of the fusion, we expected that <100% of the mutant progeny would resist the high hygromycin levels. Five of the 10 mutant progeny tested grew in hygromycin at 1500 μg/ml, whereas two others grew in hygromycin at 750 μg/ml, a level that inhibited growth of wild-type progeny. The other three mutant progeny were only as resistant to hygromycin as the wild-type progeny. Because no wild-type progeny were resistant to the high level of hygromycin and because most progeny with altered morphology were highly resistant, we conclude that the mutation in strain CH10-1 responsible for increased expression of the *con-10–hph* fusion probably also confers the conidial separation defect. From the cross of wild type by strain CH10-2, morphological mutants and wild-type progeny appeared in equal numbers. RNA isolated from mycelial cultures of seven wild-type progeny examined did not contain con-10 mRNA, whereas con-10 mRNA was detected in the nine progeny with the CH10-2 phenotype (data not shown). From the cross of wild type by CH10-3, mycelial RNA from the five wild-type progeny examined did not contain con-10 mRNA. con10 RNA was detected in 12 of the 14 aconidial segregants (data not shown). All 54 wild-type progeny from the cross of ORS-6a by CH10-4 were His$^+$, and all but one of the 83 aconidial progeny were His$^-$. This result indicates that the mutation responsible for aconidial growth was closely linked to *his-3*. con-10 RNA was not detected in mycelial RNA from the 11 wild-type progeny examined and was detected in 9 of the 10 aconidial progeny analyzed (data not shown).

Progeny from the wild type by CH10-5 cross were tested as female parents. Approximately half of the progeny served as female parents when crossed with tester strains of each mating type. Very low levels of con-10 message were detected in some progeny from this cross; a low level was present in the original mutant (Fig. 2). con-8 was used as a probe to detect strains bearing the regulatory mutation. There was no correlation between *con-8* expression and female fertility. Four of nine female sterile progeny and seven of eight wild-type progeny produced con-8 message in mycelia. We conclude that the parental mutant contained two mutations, one responsible for *con* gene expression and a second causing female sterility.

No formal test of cosegregation of the developmental mutation in strain CH10-7 with *con-10* expression was done

because it was expected that con-10 mRNA would be found in liquid "mycelial" cultures undergoing conidial development. Almost all hyperconidiating progeny were His$^-$, and all of the wild-type progeny were His$^+$. A second cross was done to verify linkage of *his-3* to the mutation conferring hyperconidiation. The single hyperconidiating His$^+$ segregant was crossed by a *his-3*-carrying strain (FGSC no. 6524). Linkage to *his-3* was observed: 17 of 17 His$^-$ progeny were wild type; 18 of 18 His$^+$ progeny were hyperconidiating. CH10-4 and CH10-7 may be allelic.

Surprisingly, all 120 progeny of the cross between strain CH10-6 and ORS-6a were morphologically wild type, as judged by spore morphology and growth rate in race tubes (data not shown). The CH10-6 strain was not genetically analyzed further. Strain CH10-8 was not examined because there was no discernible phenotype other than aberrant *con* gene expression.

Selection of Mutants with Altered Expression of *con6*. Strain CH6 contains a *con-6–lacZ* fusion integrated at the *his-3* locus and two copies of the *con-6–hph* fusion integrated ectoptically. The *con-6–hph* fusion has ≈1500 bp of the region preceding the transcription start site. Use of this strain allowed us to select mutants that were hygromycin resistant and to identify those with trans-acting mutations by screening for *lacZ* expression. The growth of strain CH6 in agar was inhibited by hygromycin at 1500 μg/ml. CH6 conidia were irradiated with UV light and plated on hygromycin agar, as described. Thirty of the most vigorous resistant colonies were screened individually for drug resistance and for expression of *con-6–lacZ*. Eight strains grew with the drug and subsequently produced variable numbers of dark blue colonies when plated on 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) agar. These presumed trans-acting mutants were designated CH6-1, CH6-8, CH6-9, CH6-10, CH6-12, CH6-13, CH6-14, and CH6-20. CH6 derivatives that were Lac$^+$ were purified by three successive rounds of plating conidia or mycelial fragments submerged in X-Gal agar and picking dark blue colonies for the next purification round. On the final plating, all mutants except CH6-1 gave uniform blue colonies. The morphology of these mutants did not change at the last purification round. Attempts to isolate a homokaryotic CH6-1 strain were unsuccessful; we believe that this strain carries a recessive-lethal trans-acting mutation.

Characterization of CH6 Trans Mutants. The eight mutant strains were compared by using the various tests listed in Table 2. All mutant strains, when grown as submerged colonies in agar, were more resistant to hygromycin than the parent. They also had 2- to 4-fold higher mycelial levels of β-galactosidase activity than the parent. These increased mycelial levels were seen repeatedly; however they were <5% of the conidial levels of the parental strain. Increased resistance to hygromycin correlated with increased β-galactosidase activity, suggesting that both increases were from mutation(s) within a single nucleus. RNA from mutant mycelia was examined for con-6 mRNA in Northern analyses, but none was detected. This observation is consistent with the fact that the β-galactosidase levels of these mutants were, at most, 4-fold greater than that of their parent. All mutant strains exhibited reduced mycelial growth rates, except CH6-1, which is thought to be heterokaryotic. Except for CH6-1, the mutant strains were morphologically distinguishable from the wild-type strain (Table 2). Three phenotypic classes were recognized. Mutants in the first class, CH6-9, CH6-10, CH6-13, and CH6-14, when grown on agar, had a conidial separation-defective phenotype. These mutants conidiate slowly and produce a dense mass of conidiophores that do not suspend readily in water and are not dispersed in air. Occasionally these strains do not produce conidia. An interesting property of mutants in this class is that their mycelial cultures have greatly increased levels of con-10 mRNA (Table 2, Fig. 3). Mutants in the second class, CH6-8 and CH6-20, do not produce conidia when grown on agar. These aconidial strains grow more slowly than their parent on agar (Table 2), and they produce increased mycelial levels of con-10 mRNA (Fig. 3). Mutants in the third class, CH6-1 and CH6-12, form normal conidia, but CH6-12 produces fewer conidia. Mycelia of CH6-1 and CH6-12 do not have increased levels of con-10 mRNA (Table 2, Fig. 3). The characteristics of CH6-1 could not be determined because this strain appears to be heterokaryotic.

Table 2. Properties of mutants derived from CH6

	Test or assay					
			mRNA[§]			
Strain*	hygR[†]	β-Gal[‡]	con-6	con-10	Growth rate[¶]	con type[‖]
CH6 (C)	>2	560	+	+	4.7	WT
CH6 (M)	0.5	3.2	−	−	4.7	WT
CH6-1	>2	8.1	−	−	4.0	WT
CH6-8	>2	12	−	+	1.3	Acon
CH6-9	>2	8.4	−	+	1.5	CSP
CH6-10	>2	8.6	−	+	2.0	CSP
CH6-12	>2	5.6	−	−	2.0	WT
CH6-13	>2	11	−	+	1.3	CSP
CH6-14	>2	7.8	−	+	1.9	CSP
CH6-20	1.8	11	−	+	1.0	Acon

*Conidia of CH6 [CH6 (C)] were assayed for β-galactosidase (β-Gal) and con-6 and con-10 mRNAs; mycelia of CH6 [CH6 (M)] and its mutants were also assayed.
†Level of hygromycin B (mg/ml) that severely inhibits growth of the strain embedded in agar.
‡β-galactosidase (β-Gal) activity (units/mg total protein).
§mRNA was (+) or was not (−) detected in Northern blots.
¶Growth rate of mycelia in mm/hr.
‖Conidiation phenotype: WT, mature conidia obtained; Acon, aconidial; CSP, conidial chain separation defective.

Crosses of the parental CH6 strain or its mutant derivatives to a wild-type strain of the opposite mating type failed to yield ascospores. Other findings suggest that this is due to the ectopic copies of the β-tubulin gene in these strains (Carl Yamashiro and C.Y., unpublished work).

DISCUSSION

In an attempt to identify the regulatory genes that control developmental formation of conidia, a translational fusion strategy was devised for selecting mutants in which a *con-6* or *con-10* fusion was expressed aberrantly—during vegetative mycelial growth. Resistant mutants were obtained, and several had trans effects on *con* gene expression. Thus, of 60 hygromycin-resistant mutants isolated in a strain bearing a *con-10–hph* fusion, eight had alterations that activated expression of the intact, normal copy of *con-10* (Table 1). These mutations also activated expression of other *con* genes and

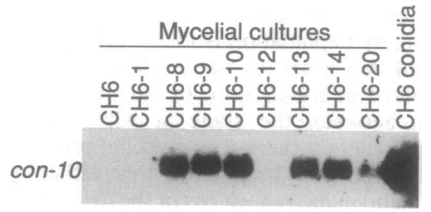

FIG. 3. Northern blots of RNA isolated from CH6 and its mutants. All strains were grown as vegetative mycelia in liquid minimal medium before RNA extraction, electrophoresis, and probing. The right lane contained RNA extracted from mature conidia. The 0.6-kb con-10 RNA was detected by hybridization with ^{32}P-labeled con-10 antisense RNA. Twenty micrograms of total RNA was loaded in each lane.

the gene *eas* (Table 1). Similarly, eight hygromycin-resistant mutants isolated in a strain with two copies of a *con-6–hph* fusion exhibited increased expression of a *con-6–lacZ* fusion (Table 2). Some of these mutants also had elevated levels of con-10 mRNA. Whether these trans-acting mutations inactivate repressors or allow premature production of transcriptional activators is unknown. In studies with a smaller set of analogous mutants that permit vegetative mycelial expression of *con-10*, the altered gene, designated *rco-1*, was shown to be recessive to its wild-type allele (C. Yamashiro, D.J.E., L. Madi, R. Brown, K. Bourland, and C.Y., unpublished work). *rco-1* has been cloned and found to be a homolog of the yeast regulatory gene *TUP1* (C. Yamashiro and C.Y., unpublished work). *TUP1* mediates a variety of regulatory events, each of which involves repression of gene expression (24–26). In view of these observations, we believe that the rco-1 protein may participate in mycelial repression of *con-10* expression.

An unexpected finding was that selection for aberrant expression of either *con-10* or *con-6* can yield mutants blocked in conidia development. Eleven of the 16 trans-acting mutations examined in this study were defective in conidiation. The block occurred most often in proconidial chain formation or in conidial separation. Our defective mutants resemble the well-known aconidial mutants *acon-2, acon-3, fld*, and *fl* and the conidial separation mutants *csp-1* and *csp-2* (27). Genetic analyses with some CH10 mutants (this study, and C. Yamashiro, D.J.E., L.M., R. Brown, K. Bourland, and C.Y., unpublished work) have shown that at least three loci are altered; none of these is in one of the known mutant loci mentioned.

Some unusual phenotypes were observed. Mutant CH10-7 forms conidia in liquid minimal medium and under the agar surface. Unlike wild type, it does not require an air interface for conidiation. Mutant CH10-6 produces an abundance of elongated spores that resemble arthroconidia. Mutant CH10-2 conidiates normally, but it forms abundant protoperithecia below the agar surface. The alteration in this mutant may have its greatest effect on the sexual cycle. Although the selection strategy used here was designed to detect genes that affect *con* gene expression primarily, as mentioned, our target *con* genes are also expressed along other spore pathways (13).

The mutants isolated in this study grow more slowly than wild type. This could indicate that the altered genes encode proteins that participate in general processes required for growth and development. Alternatively, these genes may encode proteins that are normally absent from vegetative mycelium; their formation in mycelia could interfere with some essential growth process. Because many of the mutations we have studied not only alter expression of genes that are highly expressed during conidiation but also interfere with conidial developmental, we suspect that these mutations are in regulatory genes.

Why selection for aberrant expression of *con-6* or *con-10* leads to blocks at different stages of conidiation is not known. The most likely explanation is that regulatory proteins have been altered that normally act at elements preceding many genes involved in conidiation. In our mutants there was no correlation between the stage at which conidiation was blocked and whether there was mycelial production of con-6 and con-10 mRNAs. It appears that either gene can be expressed in mycelia, regardless of the developmental block. Further characterization of these and similar mutants promises to provide insight into the regulatory molecules and events that mediate conidiation.

The process of conidiation has been studied most thoroughly in the filamentous fungus *A. nidulans* (28–30). Several key regulatory genes have been identified and cloned and shown to control progression through the various stages of conidiation (28–31). Properties of the corresponding regulatory proteins and the sites at which some of them act have been determined (32, 33). The morphological changes that occur during conidiation in *A. nidulans* and *N. crassa* differ markedly. Nevertheless, we expect that the regulatory molecules that participate in conidiation in the two fungal species are related and function similarly. Whether any mutants isolated in our study are altered in regulatory genes homologous to those identified in studies with *A. nidulans* remains to be seen.

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