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Isolation and Characterization of Genes Differentially Expressed During Conidiation of *Neurospora crassa*

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A *Neurospora crassa* genomic DNA library was screened with a cDNA probe enriched in sequences expressed in conidiating cultures. Clones were isolated that preferentially hybridized to this probe versus a second cDNA probe complementary to polyadenylated RNA isolated from mycelia. Twelve clones contained unique sequences that hybridized to 22 transcripts, 19 of which accumulated preferentially in conidiating cultures. Eight transcripts were present in higher levels in conidiating cultures than in mycelia. Eleven transcripts were detected only in conidiating cultures and first appeared at different times during the asexual cycle. We mapped genomic sequences homologous to the 11 clones by conventional crosses using restriction fragment-length polymorphisms as genetic markers. The sequences homologous to genes expressed preferentially in conidiating cultures are distributed on six of the seven chromosomes. Clones that map to the same chromosome are linked. No recombination occurred between genomic sequences homologous to three clones, suggesting that the genes contained in these clones may constitute a gene cluster.

Asexual reproduction in *Neurospora crassa* involves the differentiation of three cell structures—mycelia or vegetative hyphae, aerial hyphae, and conidia, the asexual spores. *N. crassa* can be grown under conditions that either promote vegetative growth or induce conidiation. In liquid medium, under continuous agitation, only vegetative growth occurs. However, on a solid surface or when mycelia are harvested onto filter paper and incubated under aerobic conditions, the vegetative hyphae undergo a number of morphological changes, possibly in response to aerobiosis and desiccation or nutrient limitation. The abrupt change in growth conditions upon filtration triggers the steps leading to conidiation, which then proceed in a synchronous fashion. Within 2 h vegetative hyphae begin to grow upward, perpendicular to the surface of the substrate, and differentiate into aerial hyphae. The aerial hyphae elongate, branch, and by 12 h produce large numbers of conidia by repeated apical budding. By manipulating the growth conditions in this way, homogeneous populations of cells at different stages of differentiation can be readily isolated and analyzed (1).

Studies of asexual differentiation in *Aspergillus nidulans* have shown that approximately 1,000 polyadenylated [poly(A)$^+$] RNA sequences, representing 6% of the genome, preferentially accumulate in cultures induced to conidiate (27). Several lines of evidence suggest that differential gene expression also occurs during the formation of aerial hyphae and conidia in *N. crassa*. First, a number of "phase-specific" mutants have been isolated that are defective in the differentiation of aerial hyphae or conidia, but are unaffected in vegetative growth or sexual reproduction (13, 22). Second, on two-dimensional gels of proteins synthesized during the asexual cycle, 12 polypeptides were detected in extracts of aerial hyphae or conidia that were not readily detected in extracts of mycelia. Another 14 polypeptides were present at higher concentrations in conidiating cultures than in vegetative hyphae. The results of in vitro translation experiments also suggested that there are major differences in the steady-state levels of many poly(A)$^+$ RNAs in mycelia and conidiating cultures (1).

We now report the isolation of genes induced in conidiating cultures by an approach similar to that employed by Zimmermann et al. (28) in the isolation of conidiation-specific genes in *A. nidulans*. We prepared a cDNA probe enriched in sequences expressed in conidiating cultures and used this probe to screen an *N. crassa* genomic DNA library. Clones were identified that hybridized preferentially with this probe versus a second cDNA probe complementary to poly(A)$^+$ RNA synthesized in mycelia. Eleven clones were selected that contained unique sequences that hybridized to RNAs that were induced in aerial hyphae or conidia. The pattern of synthesis of these RNAs in mycelia and conidiating cultures indicates that gene regulation during the asexual cycle is complex. Some clones correspond to genes expressed in mycelia that are induced in conidiating cultures. Others hybridize to transcripts that accumulate specifically in conidiating cultures. Transcripts in the latter catagory are first detected in conidiating cultures at different times.

The different cloned sequences were localized to specific chromosome arms. Evidence was obtained that some of the sequences are genetically linked. The organization of the cloned genes is discussed with respect to their pattern of expression during conidiation.

MATERIALS AND METHODS

Strains and culture conditions. The strain of *N. crassa* used in this work was 74-OR23-1a. Mycelia and conidiating cultures were grown as described in the accompanying paper (1).

The *Escherichia coli* strain used for propagation of the *N. crassa* genomic DNA library was LE392 (5).

Isolation of nucleic acids. *N. crassa* DNA was prepared as described by Stevens and Metzenberg (24). Poly(A)$^+$ RNA was purified by oligodeoxythymidilate-cellulose chromatography as described by Chirgwin et al. (4) from total cellular RNA isolated by the method of Reinert et al. (19).

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Screening an *N. crassa* genomic DNA library for genes preferentially expressed during conidiation. (i) Preparation of the cDNA hybridization probes. cDNA was synthesized in a reaction mixture containing 50 mM Tris-hydrochloride (pH 8.5), 40 mM KCl, 8 mM $MgCl_2$, 0.4 mM dithiothreitol, 1 mM each dATP, dGTP and TTP, 100 μg of oligodeoxythymidylic acid (Collaborative Research) per ml, 200 μCi of 3′-[α-^{32}P]dCTP (1,000 Ci/mmol; Amersham Corp.), 1 μg of heat-denatured poly(A)$^+$ RNA isolated from mycelia or conidiating cultures, and 2 U of avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc.) in a total reaction volume of 50 μl. The reverse transcriptase was kindly provided by Kevin Moore. Mixtures were incubated for 60 min at 42°C, and the reaction was stopped by adding an equal volume of 20 mM trisodium EDTA, 2 mg of salmon sperm DNA per ml, and 0.2% sodium dodecyl sulfate (SDS). Unincorporated [α-^{32}P]dCTP was separated from the cDNA by chromatography on a 2.5-ml column of Sephadex G-75 (Pharmacia Fine Chemicals). cDNAs were depleted of template RNA by hydrolysis with 0.25 M NaOH at 65°C for 5 min followed by neutralization with 2 M Tris-hydrochloride (pH 7.2).

^{32}P-labeled cDNA was synthesized from poly(A)$^+$ RNA isolated from conidiating cultures 40 h after induction. Sequences homologous to mRNAs expressed in mycelia were subtracted from the cDNA probe by a modification of the method of Timberlake (27). The ^{32}P-labeled cDNA was hybridized to a 40-fold mass excess of poly(A)$^+$ RNA isolated from mycelia to a Rot ([RNA] × time) value of 1,500. Hybrids were removed by chromatography on hydroxylapatite (Bio-Rad Laboratories). The unreacted cDNA was collected in 3 ml of 0.12 M sodium phosphate (pH 6.9)–0.1% SDS and concentrated by extraction with *n*-butanol followed by extraction with chloroform.

(ii) Preparation of filter replicas. An *N. crassa* genomic DNA library in the plasmid vector pRK9 (21) was used to transform *E. coli* LE392 to ampicillin resistance. A total of 40,000 transformants were plated directly onto sterile nitrocellulose filters (8.2-cm diameter; Schleicher & Schuell Co.) as described by Hanahan and Meselson (8) at a density of 2,000 bacteria per filter. Nitrocellulose filter replicas were prepared sequentially from each master filter. Bacterial colonies were lysed in situ, and their DNA was denatured and fixed to nitrocellulose filters as described by Grunstein and Hogness (7).

(iii) Screening the library and filter hybridization. Duplicate nitrocellulose filter replicas were prewashed and prehybridized as described by Maniatis et al. (12). The DNA on one set of filters was hybridized to the cDNA probe from which sequences complementary to mRNAs expressed in mycelia were subtracted (developmental cDNA probe). The DNA on the other set of filters was hybridized to ^{32}P-labeled cDNA synthesized from poly(A)$^+$ RNA isolated from mycelia (mycelial cDNA probe). Hybridization mixtures contained 10^6 cpm/ml of the denatured ^{32}P-labeled cDNA probe, 50% formamide, 5× Denhardt solution (5× Denhardt solution is 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin), 5× SSPE (1× SSPE is 150 mM NaCl, 9 mM NaH_2PO_4, 1.1 mM disodium EDTA), 0.1% SDS, and 100 μg of denatured salmon sperm DNA per ml. Hybridization was carried out for 12 to 16 h at 42°C. Twenty filters were washed three times for 10 min in 200 ml of 2× SSPE–0.1% SDS at room temperature and twice for 90 min in 200 ml of 1× SSPE–0.1% SDS at 68°C. Colonies that were hybridized to the developmental cDNA but not to the mycelial cDNA probe were picked, plated at low density, and screened as described above.

Gel electrophoresis and filter blot hybridization. Plasmid DNA or *N. crassa* genomic DNA was digested with restriction endonucleases and fractionated by electrophoresis in agarose gels containing 90 mM Tris-hydrochloride, 90 mM boric acid, and 3 mM disodium EDTA. DNA was transferred to nitrocellulose (Schleicher & Schuell) as described by Southern (23). Filters were prehybridized, hybridized, and washed as described by Maniatis et al. (12). Filters were hybridized with either ^{32}P-labeled cDNA or cloned DNA labeled with ^{32}P by nick translation (20). All hybridizations contained 6× SSPE, 0.5% SDS, 5× Denhardt solution, 100 μg of denatured salmon sperm DNA per ml, and 10^7 cpm of the denatured probe and were carried out for 12 to 24 h at 68°C.

Poly(A)$^+$ RNA was fractionated in agarose gels containing 2.2 M formaldehyde, 20 mM morpholinepropanesulfonic acid (pH 7.0), 5 mM sodium acetate, and 1 mM disodium EDTA as described by Lehrach et al. (11). RNA was transferred to Gene Screen (New England Nuclear) in 20× SSPE. Filters were prehybridized in 10 ml of 50% deionized formamide, 0.2% polyvinylpyrrolidone (molecular weight, 40,000), 0.2% bovine serum albumin 0.2% Ficoll (molecular weight, 400,000), 0.05 M Tris-hydrochloride (pH 7.5), 1 M NaCl, 0.1% sodium pyrophosphate, 1.0% SDS, 10% dextran sulfate (molecular weight, 500,000) and 100 μg of denatured salmon sperm DNA per ml for 6 h at 42°C. Samples of 10^7 cpm of the denatured, nick-translated DNA probe in 2 ml of the same solution minus dextran sulfate were added to the filters in prehybridization solution. Filters were hybridized for 16 to 24 h at 42°C and then washed twice for 5 min in 100 ml of 2× SSPE at room temperature, twice for 30 min in 100 ml of 2× SSPE–1% SDS at 65°C, and twice for 30 min in 100 ml of 0.1× SSPE at room temperature.

Autoradiography was performed at −80°C with Kodak XAR film and a X-ray intensifying screen.

Genetic mapping of cloned DNA fragments. The chromosomal location of each cloned genomic DNA fragment was determined by the method of Metzenberg et al. (14). Briefly, two laboratory strains of *N. crassa* carrying several conventional markers were crossed by Metzenberg et al. (14) with an unrelated wild-type strain. The laboratory strains used for mapping were *al-2*; *nuc-2*; *arg-12*; *cot-1*; *inl a* (Fungal Genetic Stock Center [FGSC] number 4411) and *un-2*; *arg-5*; *thi-4*; *pyr-1*; *lys-1*; *inl*; *nic-3*; *ars-1 a* (FGSC 4488). The wild-type strain was P538 (Mauriceville-1c A), FGSC 2225. Random progeny isolated from the crosses and scored for genetic and molecular markers were kindly provided by Robert Metzenberg. Restriction fragment-length polymorphisms were used as genetic markers to follow the segregation of genomic DNA homologous to each cloned region compared to the segregation of conventional and molecular markers in the cross. To identify restriction fragment-length polymorphisms, genomic DNAs from the parental strains were digested with various restriction endonucleases, fractionated in 0.7% agarose gels, transferred to nitrocellulose, and hybridized to individual DNA clones labeled by nick translation. Genomic DNA isolated from the progeny strains was then digested with a restriction enzyme that generates a restriction fragment-length polymorphism in a region homologous to a particular DNA clone in the parental strains. The digested DNAs were then fractionated in 0.7% agarose gels, transferred to nitrocellulose, and hybridized with the appropriate cloned DNA. Linkage group relationships were determined by calculating the percent recombination between genomic DNA homologous to the cloned DNA and conventional or molecular markers in the cross. The segregation of

trp-1 was analyzed to verify the map positions of the cloned DNA contained in Con-1 and Con-7. Since none of the parental strains contained a *trp-1* marker, the segregation of *trp-1* was analyzed by Southern hybridization as outlined above with the cloned *N. crassa trp-1* gene contained in pNC2 (21).

RESULTS

Identification of cloned DNA sequences differentially expressed during conidiation. A partial *Sau*3A *N. crassa* genomic DNA library (21) was screened with two cDNA probes representing poly(A)$^+$ RNA from mycelia and conidiating cultures to identify genes expressed preferentially during conidiation. The cDNA probe enriched in sequences expressed preferentially in conidiating cultures was prepared by a modification of the method of Timberlake (27). cDNA synthesized from poly(A)$^+$ RNA isolated from conidiating cultures was hybridized to a mass excess of poly(A)$^+$ RNA from mycelia. The hybrids were removed by chromatography on hydroxylapaptite. The unbound fraction (developmental cDNA) representing 40 percent of the starting cDNA population was used to identify conidiation-specific genes. A control probe was prepared from poly(A)$^+$ RNA isolated from mycelia.

E. coli LE392 was transformed with recombinant plasmids containing *N. crassa* genomic DNA fragments that averaged 5 kilobases (kb) in length. Nitrocellulose filters containing DNA from approximately 40,000 colonies were hybridized to the developmental and mycelial cDNA probes. The number of colonies screened with each probe represented approximately 2×10^5 kb of *N. crassa* DNA or five genome equivalents. Approximately 200 colonies gave an apparent enhanced hybridization signal with the developmental cDNA probe relative to the mycelial cDNA probe. We rescreened colonies that gave the strongest hybridization signal with the developmental cDNA probe. Our expectation was that these colonies would contain DNA sequences that corresponded to abundant mRNAs. Upon rescreening 60 of these clones, 45 hybridized preferentially to the developmental cDNA probe. To determine whether these clones contained conidiation-specific genes, plasmid DNA prepared from positive colonies was analyzed by Southern hybridization. Duplicate nitrocellulose filters containing plasmid DNA restriction fragments were hybridized to either a cDNA probe synthesized from total poly(A)$^+$ RNA isolated from conidiating cultures or from mycelia; 25 of the 45 clones hybridized preferentially to the cDNA probe complementary to poly(A)$^+$ RNA from conidiating cultures. Variation in the sequence composition of the cDNA probe synthesized for use in different experiments could explain why the remaining 15 clones were detected as positives in the second screen, but not in the third screen.

TABLE 1. Summary of clones containing sequences complementary to transcripts that accumulated preferentially in conidiating cultures

Group	Clone	No. of identical clones	Approximate transcript size (kb)
1	Con-1		0.83
2	Con-2	2	1.0
3	Con-3		2.4
4	Con-4a		2.1, 2.4
	Con-4b		2.1, 2.4
5	Con-5		1.15, 1.45, 1.75
6	Con-6	4	0.66, 1.4
7	Con-7		0.9
8	Con-8		1.0, 1.25
9	Con-9		1.0,[a] 1.1, 1.2, 2.4
10	Con-10a		0.66, 1.35
	Con-10b	3	0.66
	Con-10c		0.66
	Con-10d		0.66, 1.35
11	Con-11a	3	1.1,[a] 1.75
	Con-11b		1.1, 1.75
12	Con-12		0.66[a]

[a] Transcripts that do not accumulate preferentially in conidiating cultures.

Characterization of the clones. Two criteria were initially used to determine which of the positive clones contained unique *N. crassa* sequences: (i) the cleavage pattern generated by digestion of plasmid DNA with the restriction endonuclease *Hin*fI (data not shown) and (ii) the sizes of transcripts complementary to the cloned *N. crassa* DNA segments (Table 1). Of the 25 positive clones identified by differential hybridization, 17 had unique *Hin*fI cleavage patterns. Eight had *Hin*fI cleavage patterns identical to clones Con-2, Con-6, Con-10b, or Con-11a and were not analyzed further. Some plasmids had similar *Hin*fI cleavage patterns with comigrating restriction fragments. These clones could fortuitously contain restriction fragments of the same size or they could contain the same conidiation-specific gene on overlapping regions of DNA. Clones classified in the same group in Table 1 hybridized to one or more transcripts of the same size, exhibiting the same pattern of expression during the asexual cycle. Structural analysis of the clones within a particular group, such as those in group 4, 10, or 11, should permit us to determine whether they contain a common region specifying the same transcript(s).

Con-6 and Con-12 as well as the clones in group 10 hybridized to 0.66-kb transcripts (Table 1). However, Northern analysis established that each of the three 0.66-kb transcripts was unique. Con-12 hybridized to a transcript that accumulated in mycelia and conidiating cultures (data not shown), whereas Con-6 and the clones in group 10 hybridized to 0.66-kb transcripts expressed specifically in conidiating cultures. The 0.66-kb transcripts complementary to Con-6 and the clones in group 10 were distinguishable, since they were first detected in conidiating cultures at different times (Fig. 1). When Southern blots of restriction digests of *N. crassa* genomic DNA were probed at high stringency with each of the clones in group 10, hybridization occurred with genomic fragments of the same size, indicating that these clones contained a common sequence. This sequence was not detected in Con-6 and Con-12, each of which hybridized to a unique set of genomic fragments (data not shown).

Con-12 was the only one of the 25 clones that did not hybridize to a transcript induced in conidiating cultures. Why Con-12 was consistently detected as a positive in the screening procedure is unclear. One possible explanation is that sequences contained in Con-12 are homologous to a developmentally regulated gene that specifies a transcript that comigrates with the Con-12 transcript.

The positive clones identified by differential hybridization appear to specify at least two types of transcripts: (i) those present at higher levels in conidiating cultures than in mycelia and (ii) those detected only in conidiating cultures. We tentatively catagorized the latter as stage-specific transcripts (Table 2), although they may be present in mycelia at very low levels. Clones Con-1, Con-2, Con-3, and Con-4a specified transcripts of the first type and contained genes

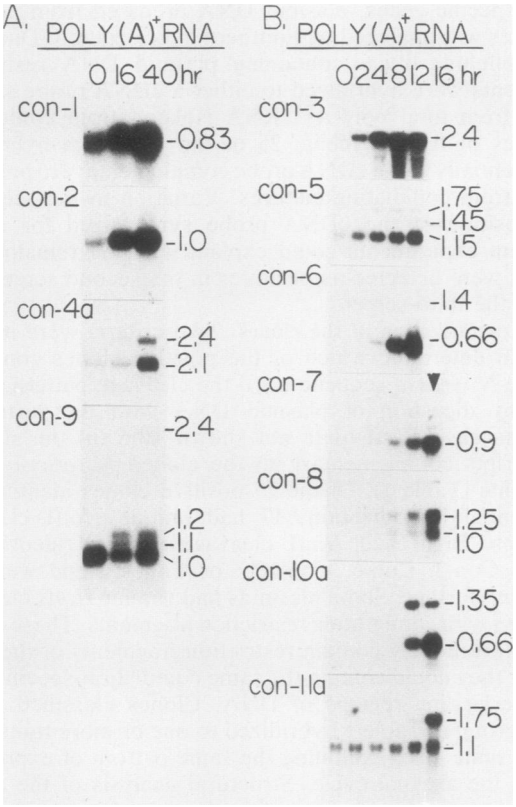

FIG. 1. Identification of transcripts complementary to genes differentially expressed in conidiating cultures. Poly(A)$^+$ RNA purified from mycelia (0 h) or conidiating cultures various times after inducing conidiation (indicated in hours at the top of each lane) was denatured, separated by electrophoresis through 1.5% agarose–formaldehyde gels, and then transferred to Gene Screen. The arrowhead indicates the 16-h RNA sample. The filters were then hybridized with plasmid DNAs radioactively labeled by nick translation. The plasmid DNA used as a probe is designated at the top left of each filter. The filters in A were prepared from standard-sized gels (12.7 by 20 cm) and contained 10 μg of poly(A)$^+$ RNA in each lane. The filters in B were prepared from minigels (5 by 7 cm) and contained 2.5 μg of poly(A)$^+$ RNA in each lane. The approximate size of each transcript, including minor transcripts visible in the original autoradiogram, is indicated to the right in kb. Size estimations are based on the migration of transcripts in standard-sized gels compared with the migration of denatured DNA fragments ranging in length from 0.42 to 1.63 kb that hybridized to vector sequences contained in the DNA probe (data not shown). The filters were exposed to Kodak XAR film for 12 to 24 h.

that exhibited different levels of induction. Con-1 and Con-2 hybridized with transcripts that increased in concentration at most severalfold in conidiating cultures compared with the levels of the Con-1 and Con-2 transcripts present in mycelia. Con-3, on the other hand, hybridized with a transcript present at low levels in mycelia that was present at relatively high levels in conidiating cultures. Con-5 and Con-6 hybridized with transcripts of both types. Con-7, Con-8, Con-9, Con-10a, and Con-11a hybridized with transcripts found exclusively in conidiating cultures, with two exceptions. The 1.0-kb transcript complementary to Con-9 decreased in abundance in conidiating cultures, whereas the 1.1-kb transcript complementary to Con-11a was expressed constitutively during the asexual cycle (Fig. 1). With a cDNA probe enriched in sequences expressed in conidiating cultures, it was possible to isolate clones such as Con-9 and Con-11a, which contained both conidiation-specific genes and genes expressed in mycelia, demonstrating the sensitivity of the screening procedure.

To characterize further the transcripts of the genes induced in conidiating cultures, positive clones were hybridized to Northern blots of poly(A)$^+$ RNA isolated from mycelia and conidiating cultures at different times after induction. Conidiation-specific genes were induced at different times during the asexual cycle (Fig. 1). Con-5 and Con-6 hybridized to transcripts 1.75 and 0.66 kb in length, respectively, first detected at 2 h. The Con-6 transcript continued to increase in concentration in conidiating cultures during the time interval examined. In contrast, the 1.75-kb transcript complementary to Con-11a was barely detectable at 12 h, but dramatically increased in concentration over the next 4 h. Con-7 and Con-8 hybridized to a total of three transcripts detected at 4 h that increased in concentration thereafter. The two transcripts complementary to Con-10a, present at low levels in 8-h cultures, were present at high levels in 12-h cultures. Table 2 presents a summary of the transcripts that hybridize to clones that contain genes expressed preferentially in conidiating cultures.

Chromosomal mapping of cloned *N. crassa* sequences that encode developmentally regulated poly(A)$^+$ RNAs. Studies of conidiation-specific genes in *A. nidulans* have shown they are clustered in the genome (16). We wished to determine whether the *N. crassa* genes specifying developmentally regulated poly(A)$^+$ RNAs that we have identified were organized in a similar fashion or distributed randomly in the genome. To address this question we mapped the chromosomal location of genomic sequences homologous to each clone with restriction fragment-length polymorphism as the basis for mapping. This method has been described by others (14) and has been used successfully to map genes of unknown function or for which there are no phenotypic markers, such as the *N. crassa* 5S RNA genes (R. L. Metzenberg, J. N. Stevens, E. U. Selker, and E. Morzycka-Wroblewska, Proc. Natl. Acad. Sci. U.S.A., in press). Restriction site polymorphism was established in genomic

TABLE 2. Summary of transcripts that hybridize to DNA clones containing genes expressed preferentially in conidiating cultures

Clone	No. of transcripts						
	Total	Not induced[a]	Induced[b]	Conidiation specific[c] detected at times (h):			
				2	4	8	12
Con-1	1		1				
Con-2	1		1				
Con-3	1		1				
Con-4	2		2				
Con-5	3		1	1		1	
Con-6	2		1	1			
Con-7	1				1		
Con-8	2				2		
Con-9	4	1	1		2		
Con-10a	2					2	
Con-11a	2	1					1

[a] Transcripts present at the same level in mycelia and conidiating cultures or at a lower level in conidiating cultures.

[b] Transcripts present at higher concentrations in conidiating cultures than in mycelia.

[c] Transcripts detected only in conidiating cultures, catagorized in terms of the hours after cultures were induced to conidiate that the transcripts were first detected.

DNA homologous to each clone in a standard laboratory strain carrying several conventional markers and an unrelated wild type strain. Random progeny from the cross (provided by R. L. Metzenberg) were classified on the basis of the alleles inherited from the parents. By comparing the segregation of specific instances of restriction site polymorphism to the segregation of conventional and previously mapped molecular markers in the cross, the sequences contained in each clone were mapped to particular linkage groups. Linkage was detected when recombination between two markers was significantly less than 50%. Linkage relationships that were ambiguous were verified by analyzing the segregation of DNA homologous to the clones in question in a second cross.

Although the number of progeny examined from each cross was too low to determine gene order with certainty, Fig. 2 illustrates the approximate map positions of each clone. The conidiation-specific genes we have isolated mapped to six of the seven linkage groups. Genomic sequences homologous to many of the clones are linked to each other (Table 3). Clones Con-5, Con-9, and Con-10b are of particular interest, since genomic DNA sequences homologous to each clone are tightly linked to *cot-1* on linkage group IV. No recombination was detected between genomic DNA corresponding to Con-5, Con-9, Con-10b, and *cot-1* in 18 progeny from one cross.

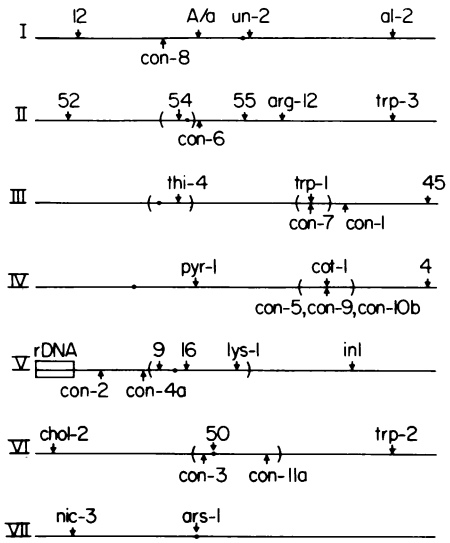

FIG. 2. Map positions of genomic DNA sequences homologous to cloned DNAs regulated during conidiation. Restriction fragment-length polymorphisms were used as genetic markers to determine the map position of genomic DNA sequences homologous to the cloned DNAs characterized in the present study and noted below each line. The segregation of restriction fragment-length polymorphisms corresponding to cloned DNAs was analyzed in the cross *al-2*; *nuc-2*; *arg-12*; *cot-1*; *inl-a*, × wild type (Mauriceville-lc-A). The segregation of sequences homologous to clone Con-11a was also analyzed in a second cross, *un-2*; *arg-5*; *thi-4*; *pyr-1*; *lys-1*; *inl*; *nic-3*; ars-1-a × wild type (Mauriceville-1c-A). The map positions of conventional markers determined previously (18) are noted above each line. The molecular markers designated by the numbers above each line refer to the 5S RNA genes mapped by Metzenberg et al. (in press). The exact order of genes enclosed in parentheses has not been determined and distances are not drawn to scale. Each line labeled with a Roman numeral represents a different chromosome. The centromere of each chromosome is shown as a solid circle.

TABLE 3. Recombination frequencies between genomic sequences regulated during conidiation and conventional or molecular markers

Clone[a]	Marker[b]	% Recombination
Con-8	A/a (I)[c]	17 (3/18)[d]
	12 (I)	19 (3/16)
Con-6	54, 55 (II)	11 (2/18)
Con-7	*trp-1* (III)	0 (0/18)
	45 (III)	39 (7/18)
Con-1	*trp-1* (III)	11 (2/18)
	Con-7	11 (2/18)
	45 (III)	28 (5/18)
Con-10b	*cot-1* (IV)	0 (0/18)
Con-9	*cot-1* (IV)	0 (0/18)
Con-5	*cot-1* (IV)	0 (0/18)
Con-2	rDNA (V)	11 (2/18)
	Con-4a	11 (2/18)
	9, 16 (V)	17 (3/18)
Con-4a	9, 16 (V)	6 (1/18)
	rDNA (V)	22 (4/18)
Con-11a	50 (VI)	12 (4/34)
	Con-3	28 (5/18)
Con-3	50 (VI)	6 (1/18)

[a] Plasmid DNAs were used to probe Southern blots of genomic DNA to analyze the segregation of homologous genomic sequences by the method of Metzenberg et al. (14).

[b] Markers include conventional markers, the 5S RNA genes denoted by numbers (Metzenberg et al., in press), and genomic DNA homologous to the clones characterized in the present study.

[c] Roman numerals designate linkage groups.

[d] Ratios indicate number of recombinants per total number of progeny analyzed from a single cross.

DISCUSSION

An *N. crassa* genomic library was screened with cDNA probes complementary to poly(A)$^+$ RNA isolated from mycelia and from conidiating cultures. Twenty-five clones were isolated that hybridized preferentially to the cDNA probe enriched in sequences complementary to genes expressed in conidiating cultures. Preliminary analysis of the clones indicate they correspond to 12 unique regions of the *N. crassa* genome. The clones hybridize to 22 transcripts, 19 of which accumulate preferentially in conidiating cultures. These results are consistent with the results of previous studies in which we demonstrated the synthesis of at least 26 proteins that are present at elevated levels in aerial hyphae or conidia (1). Whether any of the transcripts that accumulate preferentially in conidiating cultures correspond to the developmentally regulated proteins detected on two-dimensional gels (1) will require further analysis.

The transcripts detected by Northern analysis first appear at various times during the asexual cycle. Con-6 and Con-11a hybridize to transcripts first detected in conidiating cultures at 2 and 12 h, respectively. The other clones hybridize to transcripts that first appear at intermediate times. These transcripts could specify enzymes or structural proteins required for the differentiation of aerial hyphae or conidia. Alternatively, they could represent RNAs stored in conidia that specify proteins that function upon germination. It is unlikely that increased levels of these transcripts represent a general accumulation of mRNA in conidia, since other genes such as *his-3* and the gene specifying β-tubulin are not developmentally regulated (data not shown).

Six clones hybridize to multiple transcripts. Although the transcripts complementary to a single clone may correspond to different genes, we have not eliminated the possibility that

some are RNA precursors or products of differential splicing. Identification of the DNA sequences complementary to each transcript should clarify this issue.

The genes induced in conidiating cultures are distributed on six of the seven chromosomes. Two or more clones contain sequences that map to linkage groups III, IV, V, and VI (Fig. 2). In each case, the recombination frequency between sequences homologous to different clones that map to the same linkage group is significantly less than 50% (Table 3), indicating that they are linked. The sequences homologous to Con-5, Con-9, and Con-10 show the tightest linkage. These clones specify a total of nine transcripts and therefore could contain all or part of a gene cluster. Southern analyses with these clones and digests of *N. crassa* genomic DNA suggest that sequences homologous to Con-10b and Con-5 may reside on the same 12-kb *Hpa*I fragment (data not shown).

Two lines of evidence suggest that genes induced in conidiating cultures are not organized in the genome strictly according to their time of induction or pattern of expression during the asexual cycle. Clones Con-4, Con-5, Con-9, and Con-11a each hybridize to multiple transcripts that appear non-coordinately. For example, Con-11a hybridizes to a transcript that is seen late in the asexual cycle and to a second transcript whose synthesis appears to be constitutive. Furthermore, clones Con-5 and Con-10b are linked, yet they specify transcripts that appear at different times in conidiating cultures. Sequences homologous to Con-7 and Con-8, on the other hand, are on different linkage groups, but specify transcripts that appear at approximately the same time during the asexual cycle.

Transcripts specified by Con-5 and Con-6, Con-7 and Con-8, and Con-10a appear in conidiating cultures at approximately 2, 4, and 8 h after induction, respectively. An important question is whether genes activated at the same time during the asexual cycle are functionally related and induced in response to a common developmental signal. In other eucaryotic systems, distinct sequence homologies have been identified in the 5'-flanking regions of genes that are coordinately regulated. In general, the putative regulatory elements are imperfect repeats at 9 to 24 base pairs. Such sequences are present in the *Saccharomyces cerevisiae* genes involved in general amino acid metabolism (9), the heat shock genes of *Drosophila melanogaster* (2, 17), the silkworm chorion genes (10), and the tubulin genes of *Chlamydomonas reinhardi* (3). Whether homologous regions exist upstream of the transcription start sites of genes coordinately expressed during the asexual cycle in *N. crassa* remains to be determined.

Several of the genes we have isolated are linked to loci defined by mutations that affect elongation and branching of vegetative hyphae. These include "colonial" (*col*), "spreading colonial" (*spco*), "morphological" (*mo*) (6), and "colonial temperature sensitive" (*cot-1*) (15, 26) mutations (also described in reference 18). Although some of these mutations also affect conidium formation, this is probably a secondary effect of altered hyphal growth. It is unlikely that genes induced in conidiating cultures, particularly those expressed late in the asexual cycle, correspond to mutant genes that are responsible for the *col*, *cot*, *spco*, or *mo* phenotypes, since the defects in these mutants appear to affect events before aerial hypha and conidium formation. However a majority of the DNA clones we have isolated also specify transcripts that are present in mycelia; these transcripts could be essential for functions required during vegetative growth.

An important question is whether any of the genes we have isolated is required for aerial hypha or conidium formation. To assess the functions of the conidiation-specific genes we have identified, we plan to inactivate these genes by replacing them with mutant alleles generated in vitro. This approach has been used in studies on the function and regulation of cloned genes of *S. cerevisiae* (25).

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