MOLECULAR AND CELLULAR BIOLOGY, Jan. 1987, p. 427–434
0270-7306/87/010427-08$02.00/0

Vol. 7, No. 1

Position-Dependent and -Independent Mechanisms Regulate Cell-Specific Expression of the SpoC1 Gene Cluster of *Aspergillus nidulans*

BRUCE L. MILLER,† KAREN Y. MILLER,† KELLEE A. ROBERTI,‡ AND WILLIAM E. TIMBERLAKE§*

Department of Plant Pathology, University of California, Davis, California 95616

Received 11 July 1986/Accepted 13 October 1986

Many genes that are expressed specifically in the differentiating asexual spores (conidia) of *Aspergillus nidulans* are organized into clusters. We investigated the effects of altered chromosomal position on expression of a gene from the conidiation-specific SpoC1 gene cluster. The gene became deregulated when integrated at nonhomologous chromosomal sites, in that transcript levels were elevated in vegetative cells (hyphae) and variably altered in conidia. We also investigated the effects on expression of insertion of the nonregulated *argB* gene into the SpoC1 region. Levels of *argB* transcripts were markedly reduced in hyphae. The results suggest that a *cis*-acting regional regulatory mechanism represses transcription of SpoC1 genes in hyphae. They also indicate that expression of individual SpoC1 genes is modulated during conidiation by *trans*-acting factors. We propose that the two types of regulation act together to produce the major differences in transcript levels observed in hyphae versus conidia.

In the filamentous fungus *Aspergillus nidulans*, development of the multicellular asexual reproductive apparatus, the conidiophore, is characterized by the stage-specific appearance of about 1,200 different mRNAs (21). Approximately 200 of these accumulate specifically in the mature conidia (16). It is probable that conidium-specific mRNAs code for proteins that have specialized physiological or structural functions in spore differentiation or germination. The processes that control expression of *Aspergillus* conidium-specific genes are unknown.

An unusual characteristic of the conidium-specific genes is that they appear not to be randomly dispersed in the *A. nidulans* genome. Rather, the molecular analyses and statistical arguments of Zimmermann et al. (28) and Orr and Timberlake (16) indicate that the genes are often clustered. It is possible that clustering of conidium-specific genes is related to the processes responsible for their evolution or to the mechanisms regulating their expression or both.

We have subjected one gene cluster, designated SpoC1 (28), to detailed structural analysis (11, 22) in an attempt to understand the functional significance of conidium-specific gene clusters. The transcriptional organization of SpoC1 is summarized in Fig. 1. Developmentally regulated genes are clustered within a 38-kilobase (kb) region that is bounded by 1.1-kb direct repeats (RPT3). The remainder of the region consists almost entirely of unique DNA sequences. With one exception, transcription units in the central part of the cluster encode RNAs that are present at 1 to 50 copies per conidium but are undetectable in hyphae. The exceptional transcription unit, designated L8B, is not expressed in temporal or spatial coordination with those immediately adjacent to it. L8B transcripts are not detectable in hyphae or conidia but accumulate during conidiophore development. They are probably localized in the sporogenous phialide cells or their progenitor cells, the primary sterigmata. Several transcription units near the borders of the cluster encode RNAs that can be detected in hyphae but whose levels increase to some extent during conidiation. We presume that the SpoC1 transcription units constitute structural genes, because they make up open translation reading frames and they code for polyribosomal poly(A)$^+$ RNAs (11, 22; unpublished results). The biological functions of the SpoC1 gene products are unknown.

The physical organization of SpoC1 raises the possibility that there exists a regional regulatory mechanism that controls expression of many or all of the genes in the cluster (11). We have therefore investigated the effects of chromosomal position on cell-specific expression of genes from the cluster by recently developed techniques for manipulating the *A. nidulans* genome by DNA-mediated transformation (2, 13, 15, 20, 27). We describe here the results from two types of transformation experiments. In the first, expression of a SpoC1 conidium-specific gene from near the center of the cluster was examined after its relocation to different chromosomal positions. In the second, expression of the nonregulated *Aspergillus argB* gene (encoding ornithine carbamoyl transferase) was investigated after its integration into SpoC1. In both experiments, we observed significant alterations in the patterns of expression of the genes under investigation. The results indicate that cell-specific expression of the SpoC1 gene we studied is mediated by position-dependent and -independent processes. We propose a model in which two distinct regulatory mechanisms work in concert to produce the large differences in SpoC1 transcript levels observed in hyphae versus conidia. In the model, expression of the SpoC1 genes is repressed in hyphae by a regional control mechanism that is inactivated during sporulation, allowing the genes to be transcribed. Gene transcription is

* Corresponding author.
† Present address: Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843.
‡ Present address: Department of Microbiology, Oregon State University, Corvallis, OR 97330.
§ Present address: Department of Genetics, University of Georgia, Athens, GA 30602.

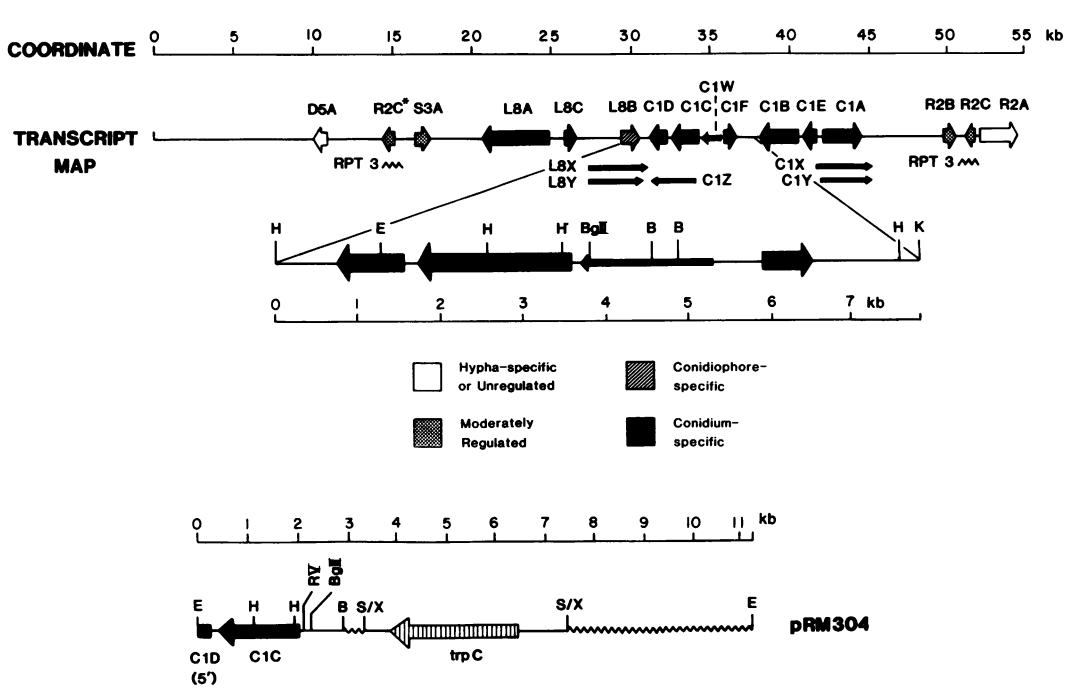

FIG. 1. (A) Transcriptional organization of the SpoC1 region. The positions and polarities of transcription units encoding prevalent RNA species are indicated by wide arrows, whereas those of transcription units encoding minor RNA species are indicated by narrow arrows. The regulatory properties of the transcription units are represented by the fill patterns indicated. The C1W transcription unit, which had not been described previously, was identified and mapped as part of this study. The scale at the top of the figure represents the arbitrary coordinate system adopted by Gwynne et al. (11). The portion of the region of greatest importance to this study is shown in expanded scale below the map of the entire cluster, and relevant restriction sites are indicated. B. Plasmid construction. Plasmid pRM304 was constructed by inserting a 3.0-kb *Eco*RI-*Bam*HI fragment containing the C1C transcription unit (▬) and a 4.1-kb *Xho*I fragment containing the *Aspergillus trpC* gene (▥) into pBR329 (∿∿). Circular plasmid DNA was used for transformations; the plasmid is shown in linear form for convenience. Symbols: H, *Hin*dIII; E, *Eco*RI; BgII, *Bgl*II; B, *Bam*HI; K, *Kpn*I; RV, *Eco*RV; S/X, *Sal*I-*Xho*I fusion.

further modulated by one or more *trans*-acting factors that interact with DNA sequences immediately adjacent to or within individual SpoC1 genes. The regional regulatory component of the model could explain why SpoC1 genes have not become dispersed in the genome during evolution.

MATERIALS AND METHODS

***Aspergillus* strains and genetic techniques.** *A. nidulans* FGSC4 was used as the wild-type strain. Strains UCD3 (*pabaA1*, *yA2*; *trpC801*,Δ*C1C*) and UCD4 (*pabaA1*, *yA1*; *argB*::*trpC*$^+$; *trpC801*) were constructed by one- or two-step gene replacement techniques as previously described (15). Standard *Aspergillus* genetic techniques were used (6, 17).

Nucleic acid isolation. DNA was isolated from hyphae by the rapid procedure of Yelton et al. (27). RNA was isolated from hyphae and spores as described previously (23). RNA concentrations were estimated by UV spectrophotometry. However, pigments contaminating the spore RNA preparations interfered significantly with these measurements. For slot blot analyses and the gel blots shown in Fig. 4 and 5, RNA concentrations were determined by hybridization analysis as follows. RNA samples were applied to nitrocellulose membranes and hybridized with an *Aspergillus* rRNA probe (pAnR2; W. Orr and W. Timberlake, unpublished results). The hybridization signals were then quantitated by densitometry, and the amount of RNA was estimated by comparison to the signals obtained from known amounts of RNA from wild-type hyphae. Parallel blots were hybridized with a nontranscribed *Aspergillus* DNA fragment to test for contamination of the RNA samples with DNA. No hybridization was detected.

S1 nuclease analysis. Low resolution S1 nuclease protection experiments (3) were done with single-stranded DNA probes as described previously (26). High-resolution experiments were done with single-stranded DNA probes prepared as described by Burke (5). The SpoC1 C1C probe was synthesized with an M13 universal sequencing primer (14) and single-stranded DNA from a bacteriophage M13 clone containing a 1.0-kb *Hin*dIII-*Bam*HI fragment from the SpoC1 region (Fig. 1A). For the *argB* probe, DNA was synthesized with an *argB* primer kindly provided by A. Upshall and G. McKnight, ZymoGenetics, Inc., and DNA from a clone containing a 1.2-kb *Bgl*II-*Hin*dIII fragment that encompasses the 5′ end of the *argB* gene (15). Protected products were analyzed by electrophoresis on sequencing gels containing molecular size standards and appropriate DNA sequence ladders.

Transformation of *A. nidulans*. *Aspergillus* strains were transformed as described previously (27). Circular plasmid DNA was used for the C1C relocation experiments. The *argB* gene was introduced between the two *Bam*HI sites occurring between the SpoC1 C1C and C1F genes as follows. A 13.3-kb *Eco*RI fragment from cluster coordinates 32.0 to 45.3 (Fig. 1A) was ligated into the *Eco*RI site of a pBR329 (7) derivative in which the *Bam*HI site had been eliminated. The resultant plasmid (pSpoC1ΔB) was cut with *Bam*HI and ligated with a 1.8-kb *Bam*HI fragment containing the *A. nidulans argB* gene plus 0.2 kb of 5′ and 3′ flanking DNA. Plasmids containing the *argB* gene in either orienta-

tion were isolated and digested with *Eco*RI. The large *Eco*RI fragments were isolated from agarose gels and used to transform *A. nidulans* UCD4 into an arginine-independent strain. In one experiment, UCD4 protoplasts were cotransformed with the purified *Eco*RI fragments and a plasmid containing the *Aspergillus oliC*31 allele with selection for oligomycin-resistant colonies. In a second approach, plasmid pSpoC1ΔB was digested with *Kpn*I and ligated with a 1.8-kb *Eco*RI-*argB* fragment via a *Kpn*I-*Eco*RI adapter. The large *Eco*RI fragments were isolated and used for transformation.

The *argB* gene was inserted into the L8B transcription unit as follows. A plasmid containing an 8.3-kb *Bam*HI fragment (Fig. 1A, coordinates 24.0 to 32.3) was cut within the L8B transcription unit with *Sma*I. A 1.8-kb *Bam*HI-*argB* fragment was ligated into this site after repair with the DNA polymerase I large fragment. The resultant plasmids containing the *argB* gene in either orientation were isolated and digested with *Bam*HI. The large *Bam*HI fragments were gel purified and used to transform UCD4 into an arginine-independent strain.

Analysis of transformants. Transformants were tested for the presence of expected genetic markers and purified by streaking conidia twice for single colonies. Integration events were deduced from the results of DNA blot analyses with several restriction endonucleases and SpoC1, *argB*, and pBR329 DNA probes. Only those transformants that yielded unambiguous DNA blot patterns were used for this study. Copy numbers of integrated plasmids were estimated by comparison of the hybridization intensities of junction and internal fragments. Insertions or deletions of ≥200 base pairs would have been detected by the procedures used. For transformant T17, the C1C gene copy number was estimated by reconstruction experiments in which two equivalent slot blots containing serial dilutions of T17 DNA were hybridized with either a ^{32}P-labeled C1C-specific probe or an *argB*-specific (single-copy) probe having identical specific radioactivities. Signal intensities were determined by densitometry.

Blot analyses. Partially depurinated DNA and denatured RNA samples were transferred from agarose gels to nylon membranes by the procedures recommended by the manufacturers. For slot blot experiments, total RNA was applied to nitrocellulose membranes with a Schleicher & Schuell slot blot apparatus and by the procedures recommended by the manufacturer. Various amounts of wild-type conidial RNA (0.002 to 1.0 μg) were loaded in one set of slots. Hyphal (5.0 μg) and conidial (0.5 μg) RNAs from the wild-type, the recipient, and the transformant strains were loaded in adjacent slots. The filters were then hybridized with the C1C-specific 0.9-kb *Hin*dIII fragment (Fig. 1A), and signal intensities were determined by densitometry. The signals obtained with strain UCD3 RNA were taken as background. The data were related to the wild-type spore level by interpolation of the standard curve. Two independently isolated RNA preparations were each analyzed twice. The experimental variability was ≤10%.

RESULTS

Patterns of C1C expression after chromosomal relocation. We chose to investigate the effects of alterations in chromosomal position on regulated expression of C1C, a spore-specific gene from near the center of SpoC1 (Fig. 1A). To discriminate between transcripts arising from the experimentally manipulated gene and its normally positioned counterpart, a strain was constructed in which a 0.9-kb *Hin*dIII fragment had been deleted from the C1C coding region (UCD3 [*yA2*, *pabaA1*; *trpC801*,*ΔC1C*]; Fig. 1A and 2) (15). This mutation caused little change in conidiophore or spore morphology as determined by light microscopy and no reduction in spore viability under laboratory conditions. Gel blots of RNA isolated from UCD3 hyphae and conidia were hybridized with the deleted *Hin*dIII fragment. There was no detectable hybridization to RNA from either cell type (data not shown).

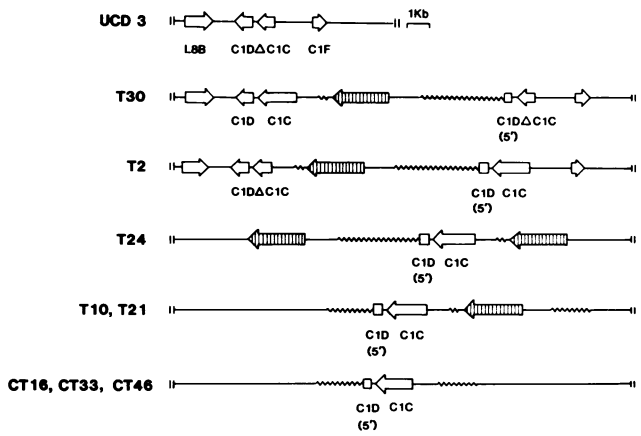

FIG. 2. Summary of C1C transformants. The structure of the relevant SpoC1 region of strain UCD3, containing a copy of the C1C transcription unit from which a 0.9-kb *Hin*dIII fragment had been removed (Fig. 1A), is represented at the top of the figure. The types of integration events in transformants were deduced from the results of DNA blot analyses with SpoC1, *trpC*, and pBR329 probes and are represented schematically in the lower portion of the figure. Symbols: ▭, SpoC1 DNA; ▮▮▮, *trpC* DNA; ∼∼∼, pBR329 DNA.

We made UCD3 tryptophan independent with plasmid pRM304 (Fig. 1B) or derivatives thereof to obtain strains having wild-type copies of C1C (including 1 kb of 5′-flanking and 0.4 kb of 3′-flanking DNA) integrated at different chromosomal sites. Two transformants (T30, T2) were obtained in which single copies of pRM304 had integrated at SpoC1 by homologous recombination with sequences on either side of the deleted *Hin*dIII fragment (Fig. 2 and Table 1). Transformant 101T3 (not shown) is the same as T30, except that it was obtained with a derivative (pRM304ΔBgB) of plasmid pRM304 that lacks the 0.8-kb *Bgl*II-*Bam*HI fragment adjacent to the 5′ end of C1C (Fig. 1B). The ability to select for these transformants showed that when integrated into SpoC1, the *trpC*$^+$ allele is expressed in hyphae at a level sufficient to allow tryptophan-independent growth. It was not possible to quantitate *trpC*$^+$ transcript levels because the *trpC801* allele of the recipient gives rise to wild-type transcript levels (unpublished results).

One transformant (T24) was obtained in which pRM304 had integrated by homologous recombination at the *trpC* locus. Transformant 101T10 (not shown) is the same as T24, except that 101T10 former was obtained by transformation with pRM304ΔBgB and has two plasmid copies integrated in tandem.

Two transformants (T10,T21) were obtained in which single copies of pRM304 had integrated at unidentified, heterologous chromosomal sites. Integration of transforming DNA at heterologous sites is common in *N. nidulans* spp. (15, 27). T28 (not shown) has two copies of pRM304 integrated in tandem at one heterologous site. T17 (not shown)

TABLE 1. Relative C1C transcript levels in hyphae and conidia

Transformant[a]	Integration site[b]	Copy no.	Relative transcript level[c]		Conidia/hyphae ratio
			Hyphae	Conidia	
Wild type	—[d]	1	<0.001	1.0	>1,000
UCD3	—	—	<0.001	<0.001	—
T30	SpoC1	1	<0.001	0.78	>780
T2	SpoC1	1	<0.001	0.73	>730
T24	*trpC*	1	0.014	0.95	68
T10	Unknown	1	<0.001	0.24	>240
T21	Unknown	1	0.021	0.19	9
T28	Unknown	2	0.068	3.6	53
T17	Unknown	~30	0.16	16.4	100
CT16	Unknown	1	0.11	1.2	11
CT33	Unknown	1	0.036	1.0	28
CT46	Unknown	1	0.030	1.6	53
101 T3	SpoC1	1	<0.001	0.55	>550
101 T4	Unknown	1	0.058	1.2	21
101 T5	Unknown	1	0.073	1.4	19
101 T10	*trpC*	2	0.084	5.0	60

[a] The properties of transformants are summarized in Fig. 2 and described in Results.
[b] Determined by DNA blot analysis with SpoC1- and *trpC*-specific probes and with plasmid pBR329. The transformants having unknown integration sites had unique junction restriction fragments, indicating that the integration events had occurred at different chromosomal sites.
[c] C1C transcript levels were quantitated by hybridizing slot blots of total RNA with a 0.9-kb *Hin*dIII fragment that had been deleted from the C1C gene in strain UCD3. Autoradiograms were analyzed by densitometry. The results are given relative to the level of C1C transcript in wild-type condia (~20 copies per cell = 1.0) (22).
[d] —, Not applicable.

has ~30 copies of the plasmid, probably integrated as two tandem arrays at heterologous sites. Transformants 101T4 and 101T5 (not shown) are similar to T10 and T21, except that the former were obtained by transformation with pRM304ΔBgB. CT16, CT33, and CT46 are similar to T10 and T21, except that the former were obtained by cotransformation with a plasmid similar to pRM304 but without the *trpC* gene and with a plasmid (pHY201; 27) containing the *trpC* gene. None of the transformants had obvious alterations in conidiophore or spore morphology or unexpected changes in nutritional requirements. All had linear growth rates of 80 to 90% of those of the wild-type strain. Germination of T28 conidia was delayed by about 33 h, resulting in the formation of small colonies.

Gel and slot blots containing RNA from hyphae and conidia of the wild-type and transformant strains were hybridized with the 0.9-kb C1C *Hin*dIII fragment. The results from gel blots are shown in Fig. 3, and those from slot blots are summarized in Table 1. No C1C transcripts were detected in wild-type hyphal RNA, even after 2 weeks of autoradiographic exposure, whereas C1C transcripts were detected in wild-type conidial RNA after several hours of exposure. Reconstruction experiments, in which slot blots containing various amounts of conidial RNA and a constant amount of hyphal RNA were hybridized with the same probe, showed that the ratio of C1C transcript levels in conidia versus hyphae was >1,000 (Table 1). Hyphal levels of C1C RNA were similarly low in transformants T30, T2, and 101T3, in which the C1C gene and associated plasmid DNA had integrated at SpoC1.

By contrast, elevated levels of C1C RNA were present in hyphae of all other transformants except T10. The levels were 1.4 to 16% of wild-type conidial levels. The lower level occurred in T24, in which pRM304 had integrated at the *trpC* locus. In 101T10, containing two copies of plasmid pRM304ΔBgB integrated at *trpC*, hyphal levels were six times higher than in T24. The highest level occurred in T17, containing ~30 copies of pRM304 integrated at heterologous sites.

C1C transcript levels were variable in conidia of the transformants. They were about 75% of the wild-type level in T30 and T2 and 55% of the wild-type level in 101T3, even though the gene had reintegrated at SpoC1. The lowest level (19%) occurred in T21, containing a single copy of pRM304 integrated at a heterologous site. The highest level (1,640%) occurred in T17, containing 30 copies of the gene. The level in T24 conidia was equivalent to that in the wild type, whereas it was five times higher in 101T10. The ratios of C1C transcript levels in conidia versus hyphae varied from >780 in T30 and T2 to 9 in T21. In the two *trpC* integrants (T24, 101T10), the ratios were nearly the same, although the absolute hyphal and conidial levels varied by five- to sixfold.

Conidial RNA from the wild-type strain and hyphal RNA from several transformants were hybridized with a single-stranded *Hin*dIII-*Eco*RV fragment complementary to the 5′ end of the C1C gene (Fig. 1B), and S1 nuclease-resistant fragments were analyzed by denaturing gel electrophoresis. Identically sized protected fragments were obtained with all

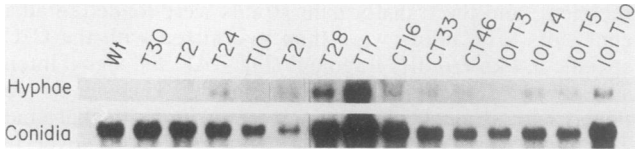

FIG. 3. Blot analysis of C1C transcripts in the wild-type and transformant strains. Total RNA was isolated from hyphae and conidia of the wild-type (Wt) and transformant strains shown in Fig. 2 and described in Results, was concentrated by LiCl precipitation, was fractionated electrophoretically under denaturing conditions, and was transferred to nylon membranes. Blots were hybridized with a ^{32}P-labeled 0.9-kb *Hin*dIII fragment (~2 × 10^8 cpm/μg) that had been deleted from the C1C transcription unit in strain UCD3 (see Fig. 2). The amounts of RNA loaded into the lanes varied to some extent (see Materials and Methods), and thus the results are simiquantitative. Quantitative results from slot blot analyses in which identical amounts of rRNA were loaded, as determined by hybridization with an rRNA probe, are given in Table 1.

the RNAs (data not shown), indicating that transcripts present in the hyphae of transformants initiated at the same sites as wild-type conidial transcripts.

Patterns of *argB* expression after integration at SpoC1. The *Aspergillus argB* gene is not developmentally regulated (26). We investigated the effects on gene expression of introducing *argB* into the SpoC1 region. An *argB* mutant *Aspergillus* strain was constructed by inserting the *trpC* gene into the ornithine carbamoyl transferase coding region (UCD4 [*yA2, pabaA1*; *argB*::*trpC*$^+$; *trpC801*]) (15). Gel blots of RNA from UCD4 hyphae and conidia were hybridized with an *argB*-specific probe. No bands corresponding to the 1.4-kb wild-type *argB* mRNA were detected (data not shown).

Strains with the *argB* gene (including 0.2 kb of the 5′- and 3′-flanking sequences) integrated into the SpoC1 region were obtained by transformation of UCD4 with linear DNA fragments containing the gene flanked by SpoC1 sequences (see Materials and Methods). In transformant TAS16-6, the gene replaced a 0.3-kb *Bam*HI fragment located on the 5′ side of the C1C gene (disrupting the minor C1W transcription unit); *argB* is in the same orientation as the C1F gene (Fig. 1A). TAS13-15 is similar, but the *argB* gene is in the opposite orientation. CTAS13-4 is the same as TAS13-15 but was obtained without selection for *argB* expression by cotransformation with a plasmid conferring oligomycin resistance (25). As controls, two transformants were obtained having single copies of the *argB*-containing plasmid integrated at the *argB* locus of UCD4 by homologous recombination with sequences on the 5′ (TAA1) or 3′ (TAA3) side of the inserted *trpC* gene. None of the transformants had obvious alterations in conidiophore or spore morphology or unexpected changes in nutritional requirements.

Gel blots of RNA from hyphae and conidia of the wild-type and transformant strains were hybridized with an *argB*-specific probe. The 1.4-kb *argB* transcript was approximately twofold more prevalent in hyphae than in conidia of the wild type (Fig. 4A). The control transformants (TAA1 and TAA3) had hyphal and conidial *argB* transcript levels equivalent to those of the wild type (data not shown). By contrast, transformant TAS16-6 produced two transcripts complementary to the probe, both of which were present at low levels in hyphae and higher levels in conidia. The larger transcript was the same size as wild-type *argB* mRNA and was transcribed from the expected DNA strand. Conidial RNA from the wild-type and transformant strains was hybridized with a single-stranded DNA fragment complementary to the 5′ end of the *argB* gene, and S1 nuclease-resistant fragments were analyzed by denaturing gel electrophoresis. The sizes of protected fragments were identical with both RNAs. We infer that the 1.4-kb transcript in TAS16-6 corresponds to wild-type *argB* mRNA and that the reduced hyphal levels of *argB* mRNA are sufficient to allow prototrophic growth. This result is consistent with the observation that reduced levels of ornithine carbamoyl transferase in *Neurospora crassa*, a closely related ascomycete, do not lead to arginine dependence (8). The smaller RNA was transcribed from the opposite DNA strand. S1 nuclease protection experiments showed that C1W transcription initiated normally. Thus, it is probable that the smaller RNA is a C1W-initiated transcript that terminates prematurely within the *argB* region.

Transformants TAS13-15 and CTAS13-14 produced 2.2-kb transcripts that were not present in hyphal RNA but were prominent in conidial RNA (Fig. 4A). A 1.4-kb transcript was present in hyphal RNA at 0.1 to 0.2 of the wild-type *argB* mRNA level but was not detectable in conidial RNA. Both RNAs were transcribed from the same DNA strand as

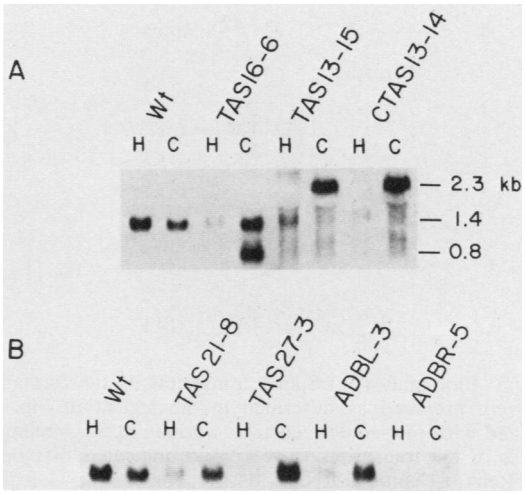

FIG. 4. Blot analysis of *argB* transcripts in the wild-type and transformant strains. (A) Blots of RNA from the wild-type (Wt) and transformant strains containing a copy of the *argB* gene integrated at SpoC1 adjacent to the 5′ end of the C1C gene were hybridized with a double-stranded DNA fragment complementary to the coding region of the gene as described in the legend to Fig. 3. The amounts of rRNA loaded into the lanes were identical in this experiment, as determined by hybridization of samples with an rRNA probe. (B) Hyphal and conidial RNAs from the wild-type and transformant strains containing the *argB* gene integrated at a *Kpn*I site adjacent to the C1B transcription unit (TAS21-8 and TAS27-3) or at an *Sma*I site within the L8B transcription unit (ABDL-3, ABDR-5) were hybridized with a double-stranded *argB*-specific probe. Symbols: H, hyphal RNA; C, conidial RNA.

that containing the C1W transcript. S1 nuclease protection experiments showed that C1W transcription initiated normally. It is thus probable that the 2.2-kb RNAs are C1W-*argB* fusion transcripts.

The *argB* gene was also introduced at two other positions in SpoC1 (in each orientation) by similar techniques. In one pair of transformants, (TAS21-8 and TAS27-3), the *argB* fragment was inserted into a *Kpn*I site near the 3′ end of the C1B transcription unit, whereas in the other pair (ADBL-3 and ADBR-5), it was introduced into the center of the L8B transcription unit (Fig. 1A). In all four transformants, hyphal levels of the *argB* transcript were reduced fivefold or more relative to that of the wild type (Fig. 4B). In the transformant containing the *argB* gene inserted into L8B in the same transcriptional polarity as that of L8B, the *argB* transcript was not detected in hyphal or conidial RNA.

Influence of transformation events on SpoC1 gene expression. Gel blots of hyphal and conidial RNA from the wild-type, UCD3, UCD4, and representative transformant strains were hybridized with probes complementary to the SpoC1 C1D, C1E, C1F, and L8C conidium-specific genes to determine whether the transformation events affected expression of SpoC1 genes other than C1C. Hyphal and conidial levels of the other SpoC1 transcripts were normal in UCD3, UCD4, and the transformants containing single integrated copies of plasmid pRM304, including T30, T2, and 101T3 (data not shown). However, hyphal levels of the C1D, C1E, and C1F transcripts were significantly elevated in T17, containing ~30 copies of C1C integrated at heterologous sites (Fig. 5). Conidial levels of the transcripts were normal. Thus, addition of numerous copies of the C1C gene to the genome at heterologous sites can affect expression of other SpoC1 genes.

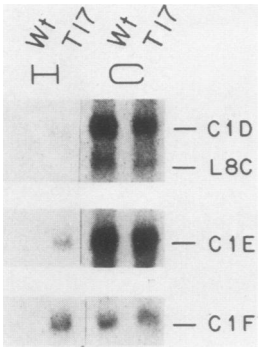

FIG. 5. Blot analysis of SpoC1 transcripts in transformant T17. Blots were prepared as described in the legend to Fig. 3 and hybridized with probes derived from various SpoC1 regions. The identities of the transcripts (Fig. 1A) are indicated. Symbols: H, hyphal RNA; C, conidial RNA.

DISCUSSION

The results presented here show that chromosomal position plays a significant role in regulating cell-specific expression of the *A. nidulans* SpoC1 C1C gene. With one exception, transformants containing the C1C gene at ectopic chromosomal sites produced elevated levels of C1C RNA in hyphae. It is not possible to calculate precisely the extent of this effect, because C1C RNA was not detected in wild-type hyphae. However, deregulated transcript levels in transformants containing single copies of the gene were 14 to 110 times greater than the detection limit (~0.02 molecules per cell) of the analytical procedure used. Similar deregulation did not occur when the C1C gene and associated plasmid sequences were reintegrated at SpoC1, indicating that the effect was not related to an intrinsic property of the plasmids used in the study. Increased hyphal transcript levels occurred in the three cotransformants examined, showing that the effect was not the result of selection for expression of a covalently linked gene. It is probable that the increases in C1C RNA levels in hyphae are the result of transcriptional activation of the gene, because correctly initiated transcripts were produced in transformants and it is unlikely that the chromosomal position of a gene would affect processing or turnover of its transcription products.

One possible explanation for these results is that within the SpoC1 region is a *cis*-acting element(s) that represses C1C transcription in hyphae and that was not included in the DNA fragment that was relocated. The results suggest that if such an element exists, it must be able to act over substantial distances, because the C1C gene was regulated normally when reintegrated at SpoC1, even though the C1C gene was separated from most of its normal 5′- or 3′-flanking sequences by 11 kb of inserted plasmid DNA in transformants T30 and T2, respectively. An alternative explanation for the results is that chromosomal relocation of the gene frequently places it under the influence of elements (for example, enhancerlike elements) that induce its expression in hyphae. If this was the case, the data would imply that such elements occur frequently in the *Aspergillus* genome, because deregulation occurred in10 of 11 transformants containing the C1C gene at presumably random heterologous chromosomal sites. They would further imply that the SpoC1 region is deficient in such elements.

The conclusion that a property of the SpoC1 region leads to reduced gene expression in hyphae is supported by the results of the *argB* relocation experiments. Insertion of this nonregulated gene at three different positions within SpoC1 in either orientation caused a fivefold or more reduction in *argB* transcript levels in hyphae. Reduced transcript levels were probably caused by reduced transcription rates, because chromosomal position is unlikely to affect posttranscriptional regulatory processes. This effect could be explained by a generalized activity of a *cis*-acting negative regulatory element or by a deficiency of *cis*-acting positive regulatory elements within the SpoC1 region. The observation that *argB* transcription was reduced in hyphae when the gene was integrated at three different sites implies that genes within the region defined by the integration sites are subject to a similar effect. Thus, it is likely that the regulatory processes acting on the C1C gene, inferred from the C1C relocation experiments, also act on other conidium-specific genes residing near the center of the SpoC1 gene cluster. These results are reminiscent of those obtained in studies of the mechanisms repressing activity of the silent mating type loci (HML and HMR) of yeast (1, 9), for which deletion of a specific *cis*-acting element leads to derepressed gene transcription. The activity of this element extends to unrelated structural genes and to a tRNA gene (4, 18), indicating that the repressive effect is the result of nonspecific interference with transcription. The results presented here are in contrast to those obtained in several studies of developmentally regulated genes in organisms as diverse as *Drosophila melanogaster* and tobacco, for which chromosomal position appears to have little or no qualitative effect on patterns of gene regulation (10, 12, 19, 24; J. K. Okamuro, D. Jofuku, and R. B. Goldberg, Proc. Natl. Acad. Sci. USA, in press).

Even though the transformants containing the C1C gene at novel chromosomal positions produced elevated hyphal levels of C1C RNA, conidial RNA levels were in all cases higher. The ratios of transcript levels in conidia versus hyphae varied from 9 to >240 in transformants containing C1C at abnormal positions, compared with ratios >1,000 in the wild-type strain. Thus, a component of the C1C regulatory system operates regardless of the chromosomal position of the gene. One possible explanation for this result is that C1C RNA is rapidly degraded in hyphae but is stabilized in differentiating conidia, leading to increased accumulation. This would not be surprising in view of the fact that C1C RNA is stored in dormant spores and is presumably not degraded until the conidia germinate. If RNA stabilization was solely responsible for the position-independent regulatory component, then elevated hyphal transcription of the C1C gene should correlate with overaccumulation of C1C RNA in conidia. This is true for some transformants but not for others. Thus, for example, T24 and 101T10 have hyphal and conidial transcript levels that differ by five- to sixfold but have the same induction ratio. By contrast, T17 and CT16 have similar, high hyphal transcript levels, but their conidial levels differ by 16-fold, with CT16 having nearly normal amounts of the C1C transcript in conidia. An alternative possibility is that the C1C-containing DNA segments used in this study have sufficient information to allow the gene to respond in *trans* to a cell-specific regulator of gene transcription. Such a regulator could act by inducing gene transcription in differentiating spores or by repressing gene transcription in hyphae. If this was the case, then the variable ratios of transcript levels in conidia versus hyphae might be explained by additional chromosome position effects.

One piece of evidence is consistent with the existence of a *trans*-acting regulator of gene expression and, furthermore, suggests that regulation occurs by repression of transcription

in hyphae. Transformant T17, containing ~30 copies of C1C integrated at heterologous sites, produces significantly elevated levels of several other SpoC1 transcripts. This effect could be explained by titration of a common repressor of SpoC1 gene activity by the multiple copies of the C1C gene or flanking DNA sequences present in T17. Titration effects have been observed in other *A. nidulans* genetic regulatory systems (M. Hynes, personal communication).

These observations lead us to propose a two-component model for regulation of SpoC1 gene expression. We suggest that transcription of genes within the cluster is repressed in hyphae by regional regulatory elements (e.g., elements that influence chromatin conformation) and by *trans*-acting factors that interact with the individual genes or adjacent DNA sequences. Repression by both mechanisms is necessary to achieve the negligible levels of transcription of SpoC1 genes observed in hyphae. Genes near the borders of the cluster might be less subject to regional control than genes near the center because of attenuation of the repressive effect, resulting in low-level hyphal expression, as is actually observed. According to the model, activation of the cluster occurs in two steps. First, the regional (position dependent) repression system is inactivated early in development, leading to a low level of transcription of the conidium-specific genes and permitting higher-level expression of the noncoordinately regulated L8B gene (Fig. 1A). Second, the gene-specific (position independent) repression system(s) is inactivated in prespore nuclei, leading to fully derepressed transcription of the conidium-specific genes. Transcription of all the genes ceases when the conidia become metabolically dormant. The model is consistent with the data presented here and with our previous observations concerning the transcription of SpoC1 genes in developmentally abnormal mutant strains (11) and can be tested further by currently available techniques for the molecular genetic manipulation of *A. nidulans*.

The position dependence of the regulation of SpoC1 genes could impede the evolutionary dispersal of the genes. If the SpoC1 gene products have activities that are detrimental to hyphal function, their translocation to other chromosomal positions would impart a selective disadvantage on the resultant strains. Conversely, translocation of genes that are active during vegetative growth to the cluster would reduce their expression, also leading to decreased fitness. The frequent occurrence of gene clusters in *A. nidulans* may indicate that position-dependent strategies for gene regulation are common in this species.

ACKNOWLEDGMENTS

We are grateful to Bob Goldberg, Norman Giles, Bob Geever, and John Harada for critical reviews of the manuscript and to our colleagues in the laboratory for many stimulating discussions. We thank Geoff Turner and Jim Ballance for providing us with an *oliC*31 plasmid.

This work was supported by Public Health Service grant GM30349 to W.E.T. from the National Institutes of Health.

LITERATURE CITED

1. **Abraham, J., K. Nasmyth, J. Strathern, A. J. S. Klar, and J. Hicks.** 1984. Regulation of mating-type information in yeast: negative control requiring sequences both 5′ and 3′ to the regulated region. J. Mol. Biol. **176**:207–331.
2. **Ballance, D. J., F. P. Buxton, and G. Turner.** 1983. Transformation of *Aspergillus nidulans* by the orotidine-5′-phosphate decarboxylase gene of *Neurospora crassa*. Biochem. Biophys. Res. Commun. **112**:284–289.
3. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1-endonuclease digested hybrids. Cell **12**:721–732.
4. **Brand, A. H., L. Breeden, J. Abraham, R. Sternglanz, and K. Nasmyth.** 1985. Characterization of a "silencer" in yeast: a DNA sequence with properties opposite to those of a transcriptional enhancer. Cell **41**:41–48.
5. **Burke, J. F.** 1984. High-sensitivity S1 mapping with single-stranded [^{32}P]DNA probes synthesized from bacteriophage M13mp templates. Gene **30**:63–68.
6. **Clutterbuck, A. J.** 1974. *Aspergillus nidulans*, p. 447–510. *In* R. C. King (ed.), Handbook of genetics, vol. 1. Plenum Publishing Corp., New York.
7. **Covarrubias, L., and F. Bolivar.** 1982. Construction and characterization of new cloning vehicles. VI. Plasmid pBR329, a new derivative of pBR328 lacking the 482-base-pair inverted duplication. Gene **17**:49–89.
8. **Davis, R. H.** 1962. A mutant form of ornithine transcarbamylase found in a strain of *Neurospora* carrying a pyrimidine-proline suppressor gene. Arch. Biochem. Biophys. **97**:185–191.
9. **Feldman, J. B., J. B. Hicks, and J. R. Broach.** 1984. Identification of sites required for repression of a silent mating type locus in yeast. J. Mol. Biol. **178**:815–834.
10. **Goldberg, D. A., J. W. Posakony, and T. Maniatis.** 1983. Correct developmental expression of a cloned alcohol dehydrogenase gene transduced into the *Drosophila* germ line. Cell **34**:59–73.
11. **Gwynne, D. I., B. L. Miller, K. Y. Miller, and W. E. Timberlake.** 1984. Structure and regulated expression of the SpoC1 gene cluster from *Aspergillus nidulans*. J. Mol. Biol. **180**:91–109.
12. **Levis, R., T. Hazelrigg, and G. Rubin.** 1985. Effects of genomic position on the expression of transduced copies of the white gene of *Drosophila*. Science **229**:558–560.
13. **May, G. S., J. A. Weatherbee, J. Gambino, M. L.-S. Tsang, and N. R. Morris.** 1985. Identification and function of beta tubulin genes in *Aspergillus nidulans*, p. 239–252. *In* W. E. Timberlake (ed.), Molecular genetics of filamentous fungi. Alan R. Liss, Inc., New York.
14. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **100B**:20–78.
15. **Miller, B. L., K. Y. Miller, and W. E. Timberlake.** 1985. Direct and indirect gene replacements in *Aspergillus nidulans*. Mol. Cell. Biol. **5**:1714–1721.
16. **Orr, W. C., and W. E. Timberlake.** 1982. Clustering of spore-specific genes in *Aspergillus nidulans*. Proc. Natl. Acad. Sci. USA **79**:5976–5980.
17. **Pontecorvo, G., J. A. Rope, L. M. Hemmons, K. D. MacDonald, and A. W. J. Bufton.** 1953. The genetics of *Aspergillus nidulans*. Adv. Genet. **5**:141–238.
18. **Schnell, R., and J. Rine.** 1986. A position effect on the expression of a tRNA gene mediated by the *SIR* genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:494-501.
19. **Spradling, A. C., and G. M. Rubin.** 1983. The effect of chromosomal position on the expression of the *Drosophila* xanthine dehydrogenase gene. Cell **34**:347–357.
20. **Tilburn, J., C. Scazzocchio, G. T. Taylor, J. H. Zabicky-Zissman, R. A. Lockington, and R. W. Davies.** 1983. Transformation by integration in *Aspergillus nidulans*. Gene **26**:205–221.
21. **Timberlake, W. E.** 1980. Developmental gene regulation in *Aspergillus nidulans*. Dev. Biol. **78**:497–510.
22. **Timberlake, W. E., and E. C. Barnard.** 1981. Organization of a gene cluster expressed specifically in the asexual spores of *A. nidulans*. Cell **26**:29–37.
23. **Timberlake, W. E., and J. E. Hamer.** 1986. Regulation of gene activity during conidiophore development in *Aspergillus nidulans*, p. 1–29. *In* J. K. Setlow and A. Hollaender (ed.), Genetic engineering, vol. 8. Plenum Publishing Corp., New York.
24. **Wakimoto, B. J., L. J. Kalfayan, and A. C. Spradling.** 1986. Developmentally regulated expression of *Drosophila* chorion genes introduced at diverse chromosomal positions. J. Mol. Biol. **187**:33–45.
25. **Ward, M., B. Wilkinson, and G. Turner.** 1986. Transformation

of *Aspergillus nidulans* with a cloned, oligomycin-resistant ATP synthase subunit 9 gene. Mol. Gen. Genet. **202**:265–270.

26. **Yelton, M. M., J. E. Hamer, E. R. de Souza, E. J. Mullaney, and W. E. Timberlake.** 1983. Developmental regulation of the *Aspergillus nidulans trpC* gene. Proc. Natl. Acad. Sci. USA **80**:7576–7580.
27. **Yelton, M. M., J. E. Hamer, and W. E. Timberlake.** 1984. Transformation of *Aspergillus nidulans* by using a *trpC* plasmid. Proc. Natl. Acad. Sci. USA **81**:1470–1474.
28. **Zimmermann, C. R., W. C. Orr, R. F. Leclerc, E. C. Barnard, and W. E. Timberlake.** 1980. Molecular cloning and selection of genes regulated in *Aspergillus* development. Cell **21**:709–715.