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The *fluffy* Gene of *Neurospora crassa* Encodes a Gal4p-Type C6 Zinc Cluster Protein Required for Conidial Development

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ABSTRACT

Neurospora crassa fluffy (*fl*) mutants are unable to produce macroconidia. We cloned the *fl* gene to determine its role in regulating conidiation. A cosmid clone containing *fl* was identified by complementation. The sequence of *fl* revealed that it encodes a Gal4p-type C6 zinc cluster protein with greatest similarity to the *N. crassa NIT4* protein that regulates genes required for nitrate utilization. Analysis of several *fl* mutant alleles demonstrated that null mutants are blocked in the budding phase of development required to produce conidiophores. *fl* mRNA is transiently induced just prior to the developmental commitment to budding growth. This timing of *fl* expression is consistent with a role for FL protein in activation of the previously characterized conidiation-specific (*con*) genes, *con-6* and *con-10*. These data suggest that FL acts as a developmentally regulated transcription factor required for conidiophore morphogenesis.

CONIDIA are the major means of dispersal for many fungi. Macroconidiation (hereafter, conidiation) in *Neurospora crassa* can be induced by exposure of the mycelium to air or by starvation of a submerged mycelium in a liquid medium lacking sufficient carbon or nitrogen (Turian and Bianchi 1972). Aerial hyphae grow by apical elongation from the mycelium within 2 hr after exposure to air. Approximately 4 hr after induction, they switch to a budding mode of growth. The transition to budding growth is characterized by the formation of minor constriction chains, which are short proconidial chains with interconidial diameters nearly as large as the diameter of the proconidia themselves. As the chain continues budding, the constrictions become more pronounced. This major constriction chain growth is first observed about 8 hr after induction (Figure 1A) (Springer and Yanofsky 1989). A double crosswall is laid down between proconidia at about 12 hr. Several hours later, the crosswalls between adjacent conidia are cleaved but the conidia are held together by a thread of material until they are dispersed by wind currents (Springer and Yanofsky 1989).

Physiological and biochemical changes associated with conidiation have been analyzed (Turian and Bianchi 1972; Weiss and Turian 1966). However, relatively few genetic loci have been found that specifically block conidiation. In 1933, Carl Lindegren was the first to identify one of these loci in a study demonstrating that phenotypes could be inherited in a Mendelian fashion in *N. crassa* (Lindegren 1933). He described this strain as having "white aerial growth and no conidia production" (Figure 1). The single gene responsible for this trait was named *fluffy* (*fl*). Since then, seven alleles of *fl* have been identified and five additional loci that specifically block conidiation have been described (Wilson 1985). *Fluffyoid* (*fld*) and *aconidiate-2* (*acon-2*) do not produce minor constriction chains and are blocked early in development (Matsuyama *et al.* 1974; Springer and Yanofsky 1989). *aconidiate-3* (*acon-3*), like *fl*, produces minor, but not major, constriction chains (Matsuyama *et al.* 1974). Two *conidial separation* (*csp-1* and *csp-2*) mutants form major constriction chains with double crosswalls but these never separate to release free conidia (Selitrennikoff *et al.* 1974). Characterization of these mutants is important to our goal of understanding the molecular genetics of conidiation.

Here we report the cloning and characterization of *N. crassa fl*. *fl* encodes a protein that resembles C6 zinc cluster transcription factors of the Gal4p class. Several aconidial mutants carry null alleles, while a less severe mutant has an allele that contains two codon changes that specify conservative amino acid substitutions. *fl* is transiently expressed during conidiation and is most abundant at the time of major constriction chain formation.

MATERIALS AND METHODS

Strains and plasmids: Unless otherwise noted, strains were obtained from the Fungal Genetics Stock Center (FGSC), Department of Microbiology, University of Kansas Medical Center. The *mutagen sensitive-23* (*mus-23*) mutant strain was provided by Dr. H. Inoue (Saitama University, Japan).

To construct pFL1, a 4.6-kilobase pair (kbp) *Apa*I-*Not*I fragment containing *fl* was subcloned into pBluescript SK$^-$ (Stratagene, La Jolla, CA) from cosmid X24:A11 of the Orbach/

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Sachs library (Orbach and Sachs 1991). The same 4.6-kb fragment was also subcloned into pCB1004 (Carroll *et al.* 1994), which contains chloramphenicol and hygromycin resistance markers. We designated this plasmid pFL2, and it was used in the complementation experiments.

Strain 74-ORS6*a* (FGSC #4200) was transformed with pFL2 and a purified hygromycin resistant transformant was crossed with strain 74-OR23-1V*A* (FGSC #2489) in order to perform repeat-induced point (RIP) mutagenesis (Selker 1990; Selker and Garrett 1988). One of the hygromycin sensitive progeny that had the *fl* phenotype was then backcrossed to 74-OR23-1V*A* to obtain a strain (LBS1) carrying the fl^{RIP} allele.

Complementation of *mus-23*: Protoplasts of the *mus-23* strain were transformed with 25 cosmid pools from the Orbach/Sachs cosmid library (Orbach and Sachs 1991), according to the procedure described by Vollmer and Yanofsky (1986). Selection for complementation was obtained by resuspending the tranformed protoplasts in 50 ml regeneration agar containing 0.025 μl/ml methyl methane sulfonate (MMS). The resuspended protoplasts were then aliquoted to five Petri dishes and the agar was allowed to solidify. Agar (20 ml) containing 2% sorbose, 0.05% fructose, 0.05% glucose, and 0.10 μl/ml MMS was then poured onto the solidified regeneration agar containing the transformed protoplasts. Transformants complemented by the *mus-23* gene are able to grow to the surface under these conditions.

Nucleic acid extraction and analysis: Genomic DNA was isolated as described previously (Vollmer and Yanofsky 1986). Southern blot analyses (Sambrook *et al.* 1989) were performed using nylon membranes (Bio-Rad, Richmond, CA). To map cosmid X24:A11 by restriction fragment length polymorphism (RFLP) analysis, we used a standard set of progeny from a cross between a Mauriceville strain and an Oak Ridge strain (Metzenberg and Grotelueschen 1995). *Bam*HI-digested chromosomal DNA generated RFLPs in the parental strains. We used *Apa*I and *Not*I fragments from both ends of the cosmid X24:A11 insert to probe a blot of *Bam*HI-digested progeny DNA. The combined fragments were labeled with α-[^{32}P]dCTP by random primed labeling (Sambrook *et al.* 1989).

RNA extraction and Northern blot analyses were performed as previously described (Madi *et al.* 1994). A 2.1-kb *Bam*HI fragment located within the coding region of the *fl* gene was used as the probe in these experiments. M. Plamann (University of Missouri, Kansas City) provided the cDNA clone of *N. crassa* actin. Probes for *con-6* and *con-10* were prepared from cDNA clones from plasmids pCON6-6 and pBW100, respectively (Madi *et al.* 1994).

DNA sequence analysis: The nucleotide sequence of *fl* has been deposited in the GenBank sequence database under accession #AF022648. The amino acid sequence of FL was used to search databases using the BLAST search algorithm (Atschul *et al.* 1990). Individual alignments with high-scoring matches were performed using the BESTFIT program (Devereux *et al.* 1984). The polymerase chain reaction (PCR) was used to amplify a 2.45-kb fragment (corresponding to nucleotides 1-2450) (Figure 2) from fl^{L}, fl^{P}, fl^{P961}, and fl^{RIP} for direct sequence analysis. A 3.3-kb PCR fragment of the fl^{Y} allele was used for direct sequencing. A second PCR amplification of the regions containing the putative mutations was performed using chromosomal DNA, and the products were sequenced to verify the mutations in each of these alleles.

Analysis of *fl* mRNA: A wild-type strain of *N. crassa* (74-OR23-1V*A*) was grown for 20 hr in Vogel's minimal medium (Davis and de Serres 1970). The culture was then washed twice with water and transferred to Vogel's minimal medium without nitrogen for an additional 8 hr. Conidiation is induced by nitrogen starvation under these conditions. The culture was harvested and RNA was extracted (Madi *et al.* 1994). A primer (5′ > ACCCGAAGAAGCAAACCCAAG < 3′) downstream of the predicted 3′ set of introns and a primer (5′ > GTACCGTAAGATTTGCAG < 3′) downstream of the predicted 5′ intron, were used to generate first strand cDNA from the RNA using a Pharmacia Biotech First Strand cDNA Synthesis Kit. The first strand products were used as the templates for PCR amplification. Primers corresponding to nucleotides 453–470 and the complement of 1514–1531 (Figure 2) were used to amplify the region spanning the putative intron in the 5′ region of the gene, and primers corresponding to nucleotides 1682–1699 and the complement of 3550–3567 (Figure 2) were used to amplify the region spanning the putative introns in the 3′ region of the gene. Amplified regions were used for direct sequencing.

To identify the 5′ end of the mRNA, a radiolabeled oligonucleotide 5′-[^{32}P]-TGCGGCATACTAGGCACACGCGTTCGGT GTTA-3′ was used for primer extension analysis. The labeled primer was co-precipitated with 20 μg total RNA (nitrogen-starved culture) and resuspended in 20 μl water. After incubating for 5 min at 65°, MMLV reverse transcriptase buffer (New England Biolabs, Beverly, MA), dNTPs (final concentration 0.5 mm), and 50 units MMLV reverse transcriptase were added. The reaction was incubated for 5 min at 42°, followed by a 5 min incubation at room temperature. An additional 50 units reverse transcriptase were added to the reaction and incubation was continued at 37° for 1 hr. The reaction was stopped by adding 1 μl 0.5 m EDTA and was then treated with RNaseA. After phenol:chloroform (1:1) extraction, the products were precipitated and processed for loading on a sequencing gel. The same primer was used to produce a sequencing ladder to estimate the sizes of the primer extension products.

Developmental Timecourse Experiment: Vogel's minimal medium (500 ml) (Davis and de Serres 1970) was inoculated with 1×10^6 conidia/ml 74-OR23-1V*A*. After incubation for 20 hr at 34° (200 rpm) the culture was harvested onto 7-cm-diameter pieces of Whatman #1 filter paper. Each mycelium filter disk was placed on Vogel's minimal media with 0.45% agar and placed in a sterile hood. Mycelial pads were quick-frozen in liquid nitrogen at the indicated times prior to RNA extraction.

RESULTS

Cloning of the *fl* gene: We employed a map-based cloning strategy to obtain *fl*. *fl* was previously mapped to the right arm of chromosome II, about 3 cM from *trp-3* (Perkins *et al.* 1982). *mus-23* was found to map between *fl* and *trp-3* (Perkins 1992). We crossed a *fl*, *trp-3;a* strain (FGSC #7200) with the *mus-23;A* strain and examined 135 random progeny. We detected 6% recombination (8/135) between *fl* and *trp-3*, 4.5% recombination (6/135) between *trp-3* and *mus-23*, and 1.5% recombination (2/135) between *fl* and *mus-23* (Figure 1B). In *N. crassa*, one map unit is equal to an average of 40 kb based on the estimated genome size, suggesting that cloning *fl* by chromosome walking from *mus-23* would be feasible.

Complementation of *mus-23* was achieved by selection for wild-type levels of resistance to MMS (see materials and methods). Three cosmid pools, X24, X22, and X11, gave rise to MMS-resistant colonies. Cosmid pool X24 was subdivided to identify a complementing subpool and the subpool was further divided until a single

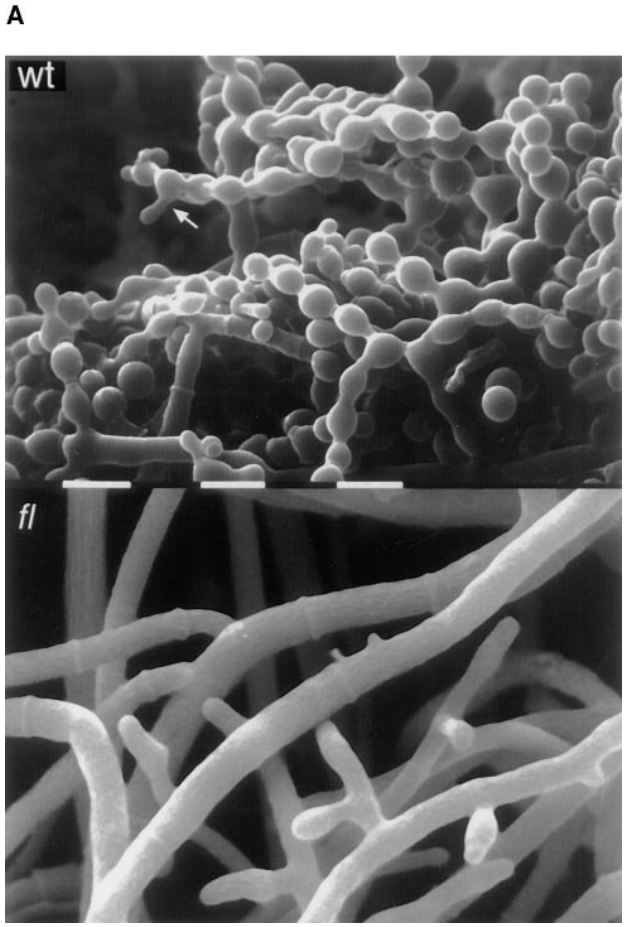
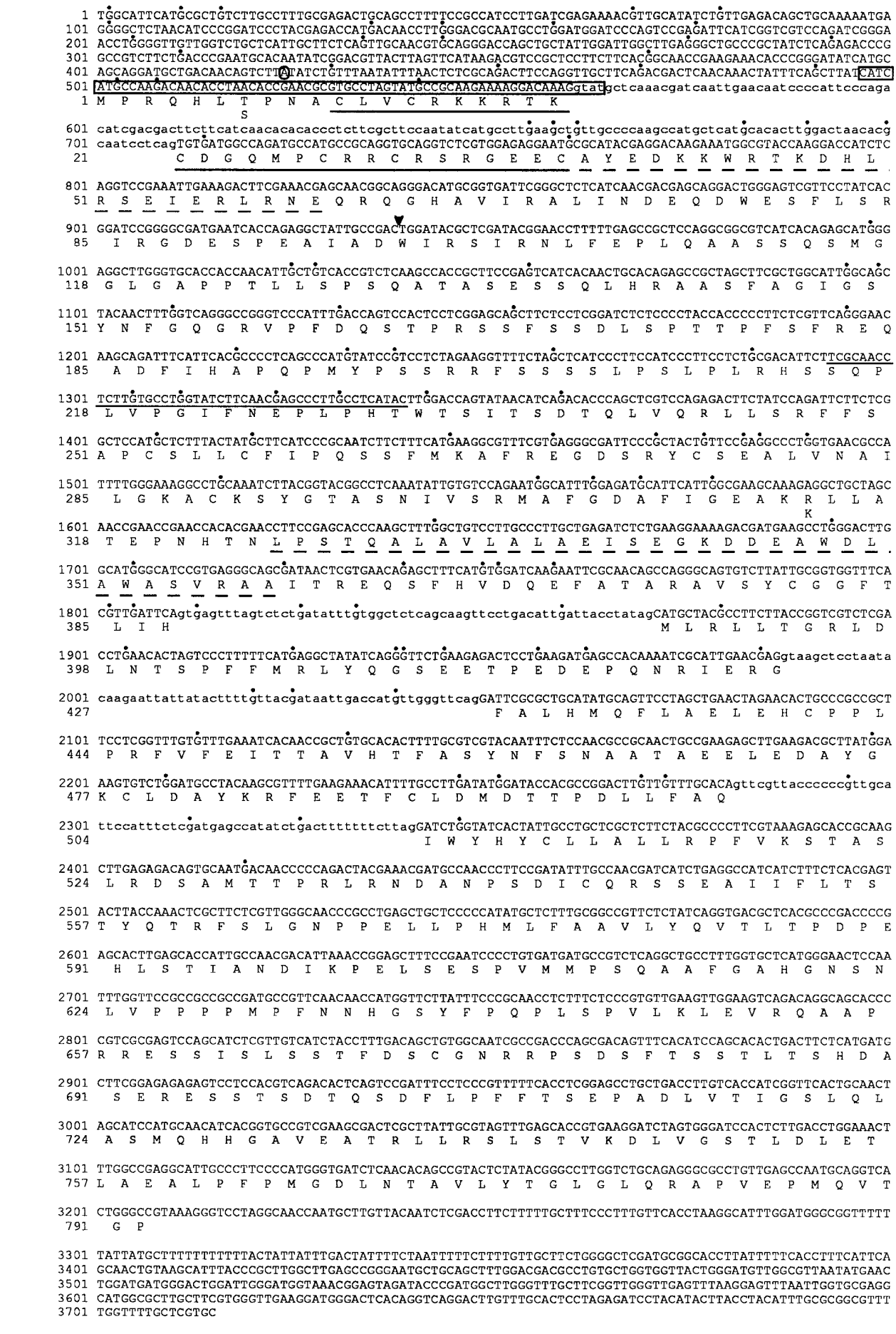


Figure 1.—Mapping of *fluffy*. (A) Scanning electron micrographs of wild-type (wt) and *fluffy* (*fl*) mutant strains. The arrow indicates a major constriction in a budding proconidial chain. Mycelial pads were harvested and exposed to air for 12 hr to induce conidiation. Scale bars = 10 μm. Micrographs courtesy of Drs. Matthew Springer and Charles Yanofsky. This phenotype was used to score progeny. (B) Genetic map of the right arm of chromosome II showing recombination distances between *fl*, *mus-23*, and *trp-3*. (C) RFLP mapping data using DNA from cosmid X24:A11 as a probe to detect an RFLP in 38 progeny from the cross of Oak Ridge (O) and Mauriceville (M) parents. The RFLP pattern is compared to the reported RFLP patterns for several markers in the *fl* region of chromosome II. The dash (—) indicates progeny not scored.

complementing cosmid, X24:A11, was isolated. The ends of the insert of X24:A11 were used as a probe for RFLP mapping of the cosmid clone (see materials and methods). These data place the *N. crassa* DNA present in X24:A11 on the right arm of chromosome II, between *bli-4* (a blue light inducible gene) and *Fsr-34* (5S RNA) (Metzenberg and Groteluenschen 1989), consistent with the location of *mus-23* (Figure 1C).

Cosmid X24:A11 was used for transformation experiments with a fl^L strain (FGSC #46), and complementation of the conidiation defect of the mutant was observed. The fl^Y mutant has reduced pigmentation and delayed conidiation. Cosmid X24:A11 also complemented the phenotype of this second allele of *fl*. A 4.6-kb *Apa*I-*Not*I fragment was subcloned and complemented the fl^L and fl^Y mutants. Subsequent analysis indicated that the *Not*I site of the 4.6-kb fragment was from the polylinker region of the cosmid vector and that the *fl* coding region was truncated at the 3′ end (position 3134 in Figure 2). The 4.6-kb *Apa*I-*Not*I fragment was used as a hybridization probe to identify additional cosmids containing *fl*. Cosmids G15:G5, G18:F4, G15:A5, and X2:D10 were identified. Cosmid G15:G5 complemented fl^L and fl^Y in transformation experiments. Direct sequencing of the 3′ end of the *fl* gene from this cosmid revealed an additional 74 nucleotides of *fl* coding region.

***fl* encodes a C6 zinc cluster protein:** Sequence analysis of a 5.2-kb region containing the complementing activity revealed the presence of a gene predicted to encode a 792 amino acid polypeptide, following the removal of four predicted introns. The first putative intron interrupts a C6 zinc cluster motif that is present near the beginning of the coding region (Figure 2). Three additional putative introns are located in the central portion of the gene. These latter three introns contain branch point sequences that differ from the consensus sequence of 5′-RCTRAC-3′ (Edelmann and Staben 1994). Instead, the corresponding sequences of these introns are CCTGAC, ATTGAC, and TCTGAC (Figure 2). It was therefore important to verify that splicing at these sites occurs *in vivo*. A cDNA clone was obtained that initiated at position 2913 and ended at position 3715 (Figure 2). There was no poly-A tail in the cDNA clone. Although we could not verify the presence of any of the predicted introns with this truncated clone, its sequence suggests that the 3′ untranslated region of *fl* mRNA is at least 500 bp in length. Reverse transcriptase-PCR products were obtained using mRNA from cultures that express *fl* (see materials and methods). Direct sequencing of PCR products spanning putative introns verified their locations. An ATG codon is located 94 nucleotides upstream of the predicted translation initiation codon of *fl*. Primer extension mapping revealed a single major 5′ end at position 424 (Figure 2), demonstrating that the potential upstream open reading frame is not present in the 5′ leader region of the predominant transcript.



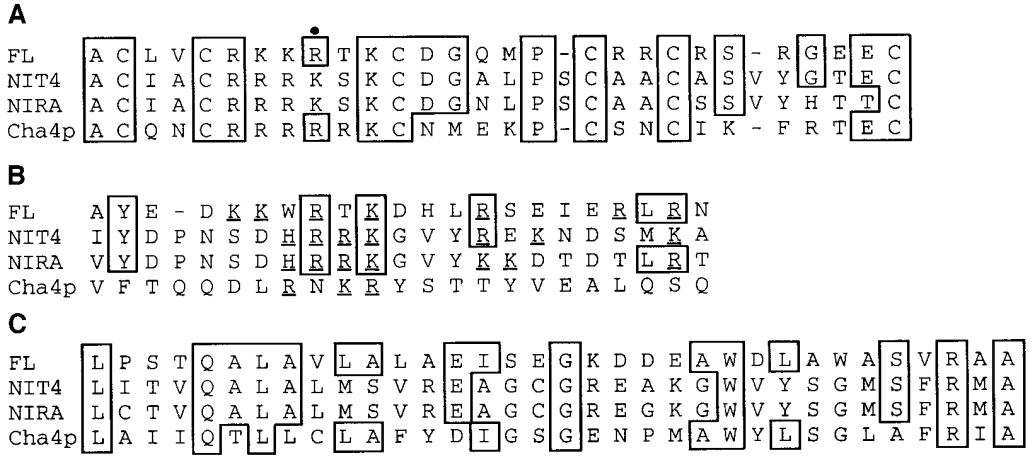


Figure 3.—Alignment of conserved regions between Gal4-type proteins FL, *N. crassa* NIT4, *A. nidulans* NIRA, and *S. cerevisiae* Cha4p. (A) The DNA binding domain. (B) The basic region. (C) The middle homology region. Arg18 is indicated by a dot above the sequence. The residue in this position is important for DNA-binding in Gal4-type proteins (De Rijcke *et al.* 1992). Residues of NIT4, NIRA, and Cha4p that are identical to FL are boxed. Basic amino acids in the basic region are underlined.

The closest match to *fl* obtained using the BLAST search algorithm (Atschul *et al.* 1990) was with another *N. crassa* gene, *nit-4*, which encodes a C6 zinc cluster transcription factor that activates expression of genes for nitrate utilization (Yuan *et al.* 1991). A comparison of the sequence of FL, NIT4 (Yuan *et al.* 1991), NIRA (the *Aspergillus nidulans* homologue of NIT4) (Burger *et al.* 1991), and Cha4p (a nitrogen-responsive regulator of serine catabolism in *Saccharomyces cerevisiae*) (Holmberg and Schjerling 1996) identified three regions of similarity common to Gal4p-type C6 zinc cluster transcription factors (Laughon and Gesteland 1984) (Figure 3). The C6 zinc cluster of FL has a spacing of cysteine residues identical to that in Cha4p (Holmberg and Schjerling 1996). A second motif conserved in Gal4-type activators is the occurrence of several basic amino acid residues that follow the C6 zinc cluster. Although this motif can be identified on the basis of charge, there is only weak sequence conservation (Figure 3B). A "middle homology" region of unknown function is conserved in FL and is equally similar among NIT4, NIRA, and Cha4p (Figure 3C).

To characterize the mutations that cause the *fl* phenotype, we amplified *fl* DNA from several mutant alleles (Figure 2, Table 1) (see materials and methods). The Lindegren allele (fl^L) was found to have a 67-bp deletion that encompasses the start codon and the first segment of the C6 zinc cluster (Figure 2). The Perkins' allele (fl^P) contains a 47-bp duplication that causes a frameshift leading to a premature stop codon (Figure 2). The fl^{P961} allele contains an insertion of a single T residue after codon 96, resulting in premature termination (Figure 2). The fl^Y allele causes a less severe phenotype and was found to contain two separate missense mutations at nucleotide positions 519 and 1591 that change the threonine of codon 7 to serine and the arginine of codon 314 to lysine (Figure 2).

To generate a null allele of *fl* that contains mutations throughout the entire coding region, we performed repeat-induced point (RIP) mutagenesis. In *N. crassa*, duplicated DNA segments may undergo RIP mutation during a sexual cross (Selker 1990; Selker and Garrett 1988). A wild-type strain bearing an ectopic copy of the pFL2 plasmid was crossed to a wild-type strain of the opposite mating type. Unlinked duplications are mutated in about half of the nuclei in a cross in RIP mutagenesis, so that about 25% of the progeny should have a mutant copy of the gene. Approximately 23% of 361 viable progeny were defective in macroconidiation. Of these, four were only partially defective in conidiation and resembled the fl^Y mutant, while the rest were aconidial. One of the hygromycin-sensitive aconidial

←

Figure 2.—Nucleotide sequence of *fl*. The *fl* gene encodes a 792 amino acid polypeptide. The coding region is interrupted by four introns indicated by lower case letters. The amino acid sequence of a C6 zinc cluster DNA-binding motif is underlined. The basic region conserved in Gal4-type zinc cluster (amino acids 38–60) and the conserved middle homology region (amino acids 325–358) are highlighted by dashed underlines. Five mutant *fl* alleles were sequenced. The 67-bp deletion in the fl^L allele is indicated by the boxed nucleotides. The 42-bp duplicated sequence of fl^P is indicated by the underlined nucleotides. The arrowhead at nucleotide position 938 indicates the site of insertion of a T residue in fl^{P961}. fl^Y contains two missense mutations that result in conservative amino acid substitutions at amino acid positions 7 and 314 as indicated. Transition mutations in the sequenced region of the fl^{RIP} allele are indicated by the dots above the nucleotides. The 5′ end of *fl* mRNA is indicated by the circled nucleotide at position 424.

TABLE 1

Summary of mutations in the *fl* alleles

Allele	Description of the mutation						
fl^{L}	Deletion of nucleotides 497–564 removes the first 20 amino acids, including the translational start codon.						
fl^{P}	Duplication of nucleotides 1293–1339 results in a frameshift after codon T230.						
fl^{P961}	Insertion of a base pair after nucleotide 938 causes a frameshift after codon D96.						
fl^{Y}	Transition mutations at nucleotides 519 and 1591 result in codon changes of threonine to serine at amino acid 7 and arginine to lysine at amino acid 314.						
fl^{RIP}	RIP allele has 105 transition mutations that result in the following codon changes in *fl*:						
	M1I[a]	S150N	G221S	E270K	A307T	M405I	G476R
	R3K	G154S	E225K	R274H	G310S	G410S	D492N
	C14Y	D161N	W231Z[b]	C276Y	W348Z	E412K	M493I
	D22N	S168N	D237N	E278K	W352Z	E416K	W505Z
	C37Y	R182K	C253Y	C289Y	V367I	E418K	M529I
	M1161	M194I	C257Y	M302I	E370K	R425Q	
	G149S	S203N	M265I	G305R	R390T	V454M	

[a] M1I indicates that methionine (M) at codon 1 is mutated to isoleucine (I).
[b] Z is the symbol used to represent a stop codon.

progeny was crossed back to wild-type and the fluffy phenotype segregated 1:1. This mutant was crossed with a fl^{L} strain, and all 125 progeny examined displayed the fluffy phenotype. We conclude that we created a new allele of *fl*, fl^{RIP}. We amplified the *fl* locus from this strain (LBS1) and sequenced this allele. RIP mutagenesis produced 105 transition mutations that resulted in 47 codon changes (Figure 2, Table 1). Key changes included altering the initiator methionine codon to an isoleucine codon and changing two of the cysteine codons of the C6 zinc cluster to tyrosine codons. In addition, tryptophans at positions 231, 348, 352, and 505 were changed to termination codons. This RIP allele is a null allele with a phenotype equivalent to fl^{L}. fl^{RIP} could be complemented by transformation with cosmid G15:G5.

***fl* is expressed transiently during development:** We induced development in a wild-type strain (74OR23-1V*A*) and harvested samples over a time-course to examine the expression pattern of *fl*. RNA from each of these samples was probed in Northern blot experiments with a 2.1-kb fragment from the *fl* coding region. *fl* expression was low at 0 and 3 hr and increased substantially 6 hr after the induction of development (Figure 4) before returning to preinduction levels by 9 hr.

con-6 is a conidiation-specific gene that is expressed at approximately 6 hr after induction of development (Roberts and Yanofsky 1989; Sachs and Yanofsky 1991). Its timing of expression precedes the transition from minor to major constriction chain formation by about 2 hr (Roberts and Yanofsky 1989; Sachs and Yanofsky 1991). The timing of *con-6* expression is coincident with increased expression of *fl*. *con-10* is typically expressed 8–10 hr after induction of development and its expression coincides with initiation of major constriction chain formation (Sachs and Yanofsky 1991). In this experiment a low-level of *con-10* mRNA was detected

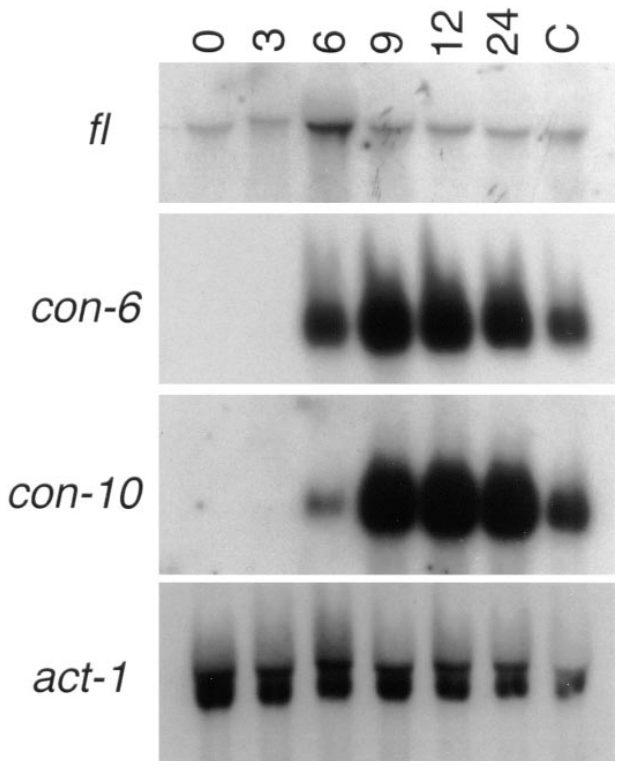


Figure 4.—Northern blot analysis of *fl* expression during development. Panels are northern blots probed with *fl*, *con-6*, *con-10*, and *actin* (*act-1*). A wild-type culture was grown in minimal medium for 20 hr and harvested onto separate filter paper circles. The harvested mycelial pads on the filters were placed onto agar minimal medium and incubated in the air for 0, 3, 6, 9, 12, and 24 hr before harvesting for RNA extraction. Each lane contained 20 μg total RNA. *act-1* was used as a control for mRNA loading. *fl* and *act-1* blots were exposed for 3 days. *con-6* and *con-10* blots were exposed for 4 and 12 hr, respectively.

at 6 hr and expression reached maximal levels at 9 hr (Figure 4).

DISCUSSION

fl has long been thought to be an important regulator of conidial development in *N. crassa*. To further our understanding of the conidiation process we isolated *fl* and began to examine its role as a regulator of conidiation. Several lines of evidence prove that we have cloned the *fl* gene. Complementation of the aconidial phenotype of a fl^{L} strain and the delayed conidiation and pigmentation phenotypes of a fl^{Y} strain was accomplished with the cloned gene. RFLP mapping of the cloned gene placed it in the same region of the genome as the *fl* locus. RIP inactivation of the cloned gene produced a null mutant that phenotypically resembles other aconidial *fl* mutants and no recombination between fl^{RIP} and fl^{L} was observed in sexual crosses. Sequence data of amplified *fl* alleles from three mutants revealed a deletion, a duplication, and an insertion in the gene that would produce a defective protein or no protein at all. The fl^{Y} allele contained two conservative amino acid substitutions consistent with the less severe phenotype caused by this allele.

The main features required for proper function of Gal4p-type proteins are thought to be the C6 zinc cluster, the basic dimerization region, the middle homology region, and an acidic activation region (Schjerling and Holmberg 1996). The sequences of the four alleles of *fl* provide initial information about the sequence requirements for FL activity. The truncated proteins predicted from the fl^{P} and fl^{P961} alleles appear to be null alleles similar in phenotype to fl^{L} and fl^{RIP}. Thus, the DNA-binding domain and basic regions are not sufficient for FL function. Complementation of *fl* null alleles by the pFL2 clone that is truncated at the C terminus suggests that there is flexibility in the sequence requirements of this portion of the protein. This region does not contain any of the four important regions described. However, the two conservative amino acid changes in the fl^{Y} allele are not located in any of the highly conserved domains, yet cause delayed conidiation and weak pigmentation. The threonine to serine or arginine to lysine mutations may have subtle effects on protein function or stability. Another possibility is that additional mutations exist in the unanalyzed regions of the promoter that could alter *fl* expression and cause the fl^{Y} phenotype.

The precise function of the middle homology region of the Gal4-type proteins has not been determined. An internal region of Gal4p, including the middle homology region, is involved in glucose repression of Gal4p activity (Stone and Sadowski 1993). In addition, when the middle homology region of Leu3p was largely deleted, it was able to activate *LEU2* regardless of the presence of the co-activator α-isopropylmalate (Zhou *et al.* 1990). These data imply that the middle homology region is involved in responding to pathway-specific signals such as glucose or α-isopropylmalate levels (Burger *et al.* 1991; Yuan *et al.* 1991) or, in the case of NIT4 and NIRA, nitrogen status.

FL displays the highest sequence similarity to NIT4 and NIRA, regulators of nitrate assimilation in *N. crassa* and *A. nidulans*, respectively. Interestingly, the first nitron of *fl* and the first intron of *nirA* are located in the same position in the C6 zinc cluster domain (Figure 2) (Burger *et al.* 1991). FL also has similarity to Cha4p, a yeast factor that regulates catabolism of serine in the absence of alternative nitrogen sources (Holmberg and Schjerling 1996). Many of the members of the Gal4p family of proteins are pathway-specific regulators of metabolism. FL appears to be a specific regulator of conidiation; however, it is tempting to speculate that FL activity could respond to nitrogen status to modulate the abundance or timing of conidia production. *fl* mRNA was detected in nitrogen-starved cultures that were producing conidiophores (data not shown).

Expression of *fl* during synchronized conidiation occurs approximately 6 hr after exposure of the mycelium to air. The timing of *fl* induction is consistent with a role in initiating major constriction growth in response to earlier developmental cues. In wild-type cells, the transition to major constriction chain growth occurs between 6 to 9 hr after induction of development. *fl* null mutants initiate the early stages of budding growth and can form short minor constriction chains but do not initiate major constriction chain budding. Minor constriction chains are capable of reverting to hyphal growth and are not developmentally committed to budding growth (Springer and Yanofsky 1989).

Several conidiation-specific genes of unknown function have been examined for expression patterns in the wild-type and in developmental mutants. *con-8* is expressed early in development and is also induced during aerial growth of a *fl* mutant (Roberts and Yanofsky 1989). *con-6* is induced just prior to major constriction chain growth, and its expression is reduced to 5–25% of wild-type levels in a *fl* mutant (Roberts and Yanofsky 1989)). *con-10* is expressed during major constriction chain growth and is not induced in a *fl* mutant (Roberts and Yanofsky 1989)). The timing of *fl* expression relative to *con-6* and *con-10* is consistent with a direct role for FL in activating these genes during major constriction chain formation. Alternatively, FL may indirectly control *con* gene expression by activation of a developmental program for major constriction chain growth.

N. crassa and *A. nidulans* serve as important model systems for the study of fungal genetics. Morphologically, the structures of the *N. crassa* and *A. nidulans* conidiophores differ greatly. Recently, a *N. crassa* homologue of the *A. nidulans flbD* gene (Wieser and Adams 1995) was cloned and characterized (Shen *et al.* 1998). Although the *N. crassa* homologue complements the

delayed conidiation phenotype of an *A. nidulans flbD* mutant, a corresponding mutant produced in *N. crassa* displayed no defect in macroconidiation or microconidiation. *fl* is the first specific regulator of conidial development to be analyzed from *N. crassa* and it is not homologous to any of the known regulatory genes governing conidiation in *A. nidulans* (Adams 1995). These findings suggest that the genetic pathways controlling conidial development differ in these two fungal species.

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