Isolation and Molecular Characterization of the *Aspergillus nidulans wA* Gene

María E. Mayorga and William E. Timberlake

Department of Genetics, University of Georgia, Athens, Georgia 30602

Manuscript received November 22, 1989
Accepted for publication March 9, 1990

ABSTRACT

The walls of *Aspergillus nidulans* conidia contain a green pigment that protects the spores from damage by ultraviolet light. At least two genes, *wA* and *yA*, are required for pigment synthesis: *yA* mutants produce yellow spores, *wA* mutants produce white spores, and *wA* mutations are epistatic to *yA* mutations. We cloned *wA* by genetic complementation of the *wA3* mutation with a cosmid library containing nuclear DNA inserts from the wild-type strain. The *wA* locus was mapped to an 8.5–10.5-kilobase region by gene disruption analysis. DNA fragments from this region hybridized to a 7500 nucleotide polyadenylated transcript that is absent from hyphae and mature conidia but accumulates during conidiation beginning when pigmented spores first appear. Mutations in the developmental regulatory loci *brlA, abaA, wetA* and *apsA* prevent *wA* mRNA accumulation. By contrast, *yA* mRNA fails to accumulate only in the *brlA*$^-$ and *apsA*$^-$ mutants. Thus, the level of *wA* transcript is regulated during conidiophore development and *wA* activation requires genes within the central pathway regulating conidiation.

CONIDIA of the ascomycetous fungus *Aspergillus nidulans* contain in their walls a dark green pigment that is not present in other cell types. Pigment is produced as spores mature (LAW and TIMBERLAKE 1980) and confers resistance to ultraviolet light (WRIGHT and PATEMAN 1970; ARAMAYO, ADAMS and TIMBERLAKE 1989). The production of spore pigment requires expression of at least two genes, *yA* and *wA*. *yA* mutants produce yellow spores, *wA* mutants produce white spores, and *wA, yA* double mutants produce white spores (PONTECORVO *et al.* 1953; CLUTTERBUCK, 1972). The product of *yA* is a *p*-diphenol oxidase, or laccase, that converts a yellow pigment precursor to the mature green form (CLUTTERBUCK, 1972; LAW and TIMBERLAKE 1980; KURTZ and CHAMPE 1982). The product of *wA* is unknown.

The observations that *wA* mutants are not deficient in *yA*-encoded laccase (CLUTTERBUCK 1972) and that *wA* mutations are epistatic to *yA* mutations (PONTECORVO *et al.* 1953) are consistent with the hypothesis that the product of *wA* catalyzes the synthesis of a yellow pigment intermediate from a colorless precursor (KURTZ and CHAMPE 1982). However, ultrastructural and biochemical studies of *A. nidulans* conidial walls have shown that *wA* mutants lack some structural wall components present in wild-type conidia, including melanin and an electron dense outer layer containing α-1,3-glucan (OLIVER 1972; CLAVERIE-MARTIN, DIAZ-TORRES and GEOGHEGAN 1988). This observation raises the possibility that the product of *wA* is involved in the synthesis of structural wall components. These components could be required for deposition or localization of the mature green pigment.

We wish to determine the mechanisms regulating expression of *wA* and *yA* during conidiophore development. *yA* has been cloned and its expression shown to be regulated at the level of mRNA accumulation (YELTON, TIMBERLAKE and VAN DEN HONDEL 1985; O'HARA and TIMBERLAKE 1989). In this paper, we describe the physical isolation and preliminary characterization of the *wA* gene. Our results show that *wA* codes for a large polyadenylated transcript capable of encoding a protein of up to 250 kDa. The *wA* transcript is undetectable in vegetative cells, accumulates during conidiation, and is absent from mature spores. Thus, it is likely that *wA*, like *yA*, is specifically expressed in the sporogenous phialide cells.

MATERIALS AND METHODS

Aspergillus strains, growth conditions and genetic techniques: *A. nidulans* strain NK002 (*pabaA1, yA2; wA3; veA1, trpC801*) was constructed by crossing G324 (*yA2; wA3; sC12, ivoA1, methH2, argB2, galA1; veA1*; Glasgow Stock Collection) and FGSC237 (*pabaA1, yA2; veA1, trpC801*; Fungal Genetics Stock Center) and used as transformation recipient for identification of *wA3*-complementing clones. Strain NK002 was used to construct diploids with a white-spored strain (TNK15-4) made by transformation of PW1 (*biA1; argB2; methG1; veA1*; P. WEGLENSKI, Department of Genetics, University of Warsaw, Poland) with pNK15. Strain TMS003 (*pabaA1, yA2; ΔargB::trpCΔB; veA1, trpC801*) was constructed by MARY STRINGER in our laboratory and used as the transformation recipient for the *wA* disruption analysis. The white-spored strain TNK22-1 (*pabaA1, yA2; wA::argB; ΔargB::trpCΔB; veA1, trpC801*) was made by transformation of TMS003 with pNK22 and crossed with

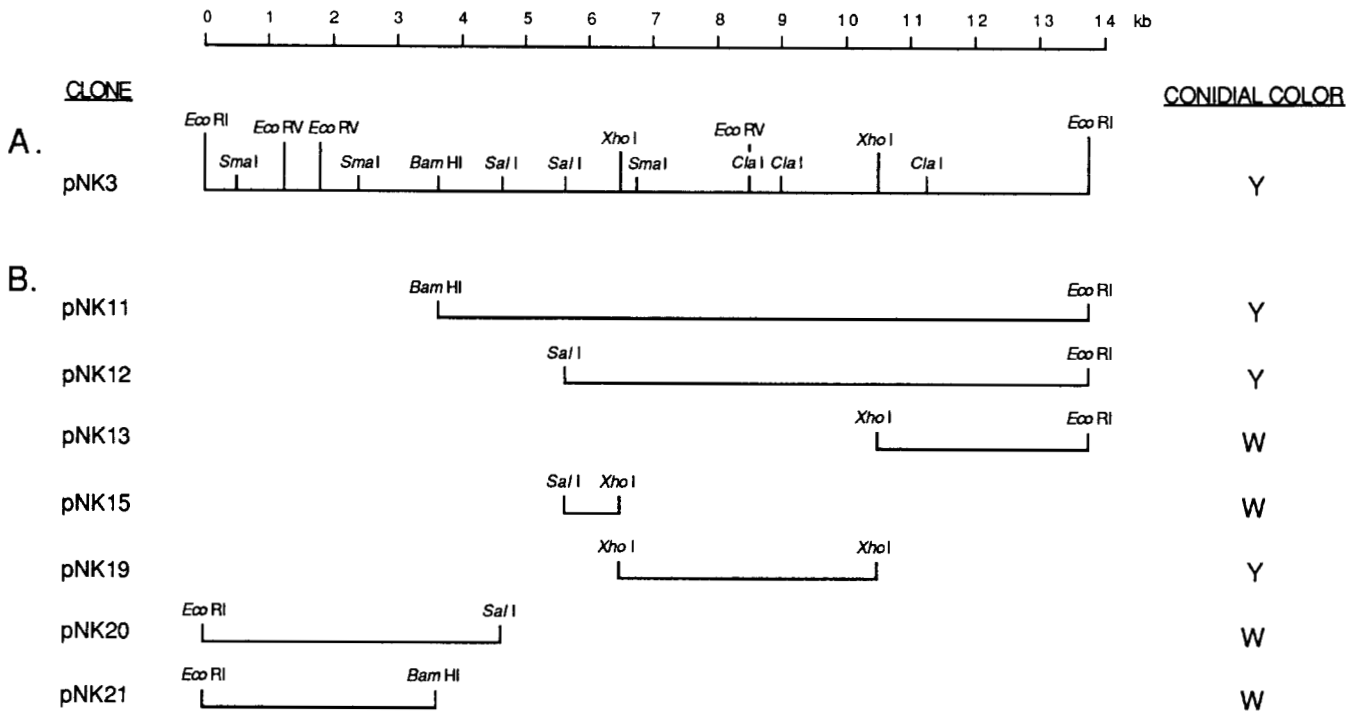


FIGURE 1.—Localization of the *wA3* complementing activity. (A) A 13.8-kb *Eco*RI fragment that complemented the *wA3* mutation was subcloned from CosNK002 as pNK3 and the indicated restriction sites were mapped. (B) Restriction fragments were subcloned from pNK3 and tested for their ability to complement the *wA3* mutation in *A. nidulans* strain NK002 by cotransformation. Complementation in this white-spored (W) strain gave rise to yellow-spored (Y) colonies because NK002 also carries the *yA2* mutation.

FGSC357 (*biA1*; *wA3*) to show linkage of cloned region to *wA3*. Strains AJC7.1 (*biA1*; *brlA1*), GO1 (*biA1*; *abaA1*), GO241 (*biA1*; *wetA6*), and AJC1.1 (*biA1*; *apsA1*) were provided by JOHN CLUTTERBUCK, Department of Genetics, Glasgow University, Scotland. *brlA1* strains initiate conidiation normally forming conidiophore stalks, but stalks grow indeterminately. *abaA1* strains form primary sterigmata (metulae) that produce functionally deranged phialides that proliferate instead of forming G_1-arrested conidia. *wetA6* strains produce normal conidiophores at permissive temperature (30°) but produce autolytic conidia at restrictive temperature (37°). *apsA1* strains produce sterigmata but nuclei fail to migrate into them, inhibiting phialide and conidium formation. Strain FGSC26 (*biA1*; *veA1*) was used for RNA isolations. Strains were grown in appropriately supplemented minimal medium with NO_3^- as nitrogen source (KÄFER 1977).

For the developmental time course experiment, FGSC26 was inoculated at a density of 3.5×10^5 conidia/ml into supplemented minimal medium containing ampicillin and streptomycin at 25 μg/ml and shaken at 300 rpm at 37° for 24 hr. Cells (100 ml) were harvested onto 10 cm Whatman No. 1 filter papers by vacuum filtration. Each filter paper was transferred to a Petri dish containing a monolayer of 3-mm glass beads and 18 ml of supplemented minimal medium. Covers were replaced, and the dishes were incubated at 37°. Samples (four Petri dishes per time point) were taken at 0, 3, 6, 8, 10.5, 12, 15, 20.5, 25, 30 and 40 hr.

Standard *A. nidulans* genetic (PONTECORVO *et al.* 1953; CLUTTERBUCK 1974) and transformation (YELTON, HAMER and TIMBERLAKE 1984; TIMBERLAKE *et al.* 1985) techniques were used. The *wA3*-complementing cosmid CosNK002 was isolated from an *A. nidulans* genomic library in pKBY2 as described by YELTON, TIMBERLAKE and VAN DEN HONDEL (1986).

Nucleic acid isolation and gel blots: DNA and RNA were isolated as described by TIMBERLAKE (1986). RNA was electrophoretically fractionated in formaldehyde-agarose gels and transferred to nylon membranes (Hybond-N, Amersham Corp., Arlington Heights, Illinois). DNA fragments were labeled with ^{32}P by nick translation and hybridized to filters according to the procedures recommended by the membrane supplier. The transcriptional polarity of *wA* was determined by using pNK15 as template to make radiolabeled RNA hybridization probes by *in vitro* transcription with T3 or T7 RNA polymerase.

Plasmid constructions: The following plasmids were constructed by using standard recombinant DNA techniques (AUSUBEL *et al.* 1987):

pNK3: A 13.8-kb *Eco*RI fragment from CosNK002 containing the *wA3* complementing activity was inserted into the *Eco*RI site of pUC13Cm^r (polylinker sites: *Hin*dIII, *Pst*I, *Sal*I, *Xba*I, *Bam*HI, *Sma*I, *Sst*I, *Eco*RI, provided by KENN BUCKLEY, Department of Genetics, University of Georgia, Athens.)

pNK11: pNK3 was digested with *Bam*HI and religated, deleting the 3.5-kb *Eco*RI–*Bam*HI fragment and a portion of the vector polylinker.

pNK12: pNK3 was digested with *Sal*I and religated, deleting the 5.5-kb *Eco*RI–*Sal*I fragment and a portion of the vector polylinker.

pNK13: pNK3 was digested with *Sal*I and *Xho*I and religated, deleting the 10.5-kb *Eco*RI-*Xho*I fragment and a portion of the vector polylinker.

pNK15: A 900-bp *Sal*I-*Xho*I fragment from pNK12 was ligated into the *Sal*I-*Xho*I sites of pBluescript KS M13$^+$ (Stratagene, San Diego, California).

pNK19: A 4-kb *Xho*I fragment from pNK12 was ligated into the *Xho*I site of pIC19-H (MARSH, ERFLE and WYKES 1984).

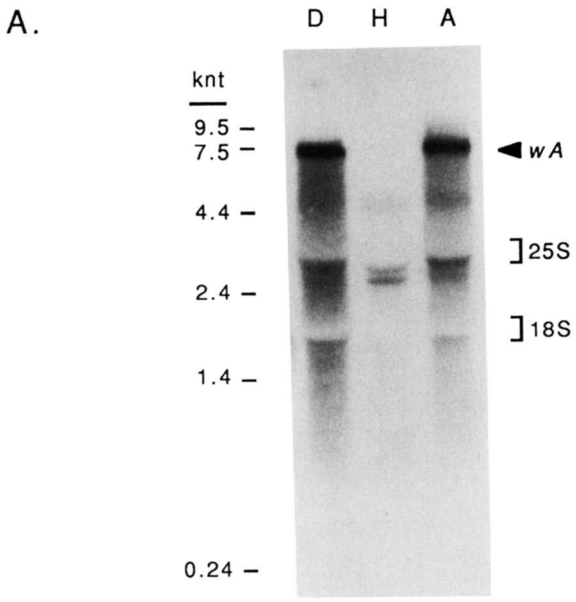
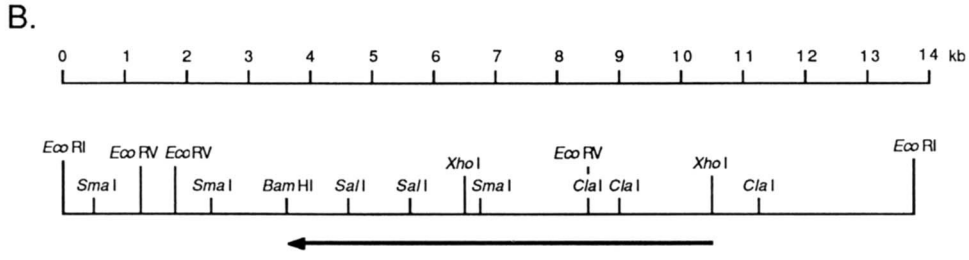


FIGURE 2.—RNA blot analysis of the *wA* region. (A) RNA was isolated from conidiating cultures (D), hyphae (H), or *abaA*-induced hyphae (A), fractionated in a denaturing agarose gel, and a blot was hybridized with radiolabeled pNK3 DNA. Locations of molecular weight standards and *A. nidulans* rRNAs were determined by ethidium bromide staining of the gel. (B) Restriction map of the pNK3 insert showing the extent and direction (arrow) of *wA* transcription.



pNK20, 21: The 13.8-kb *Eco*RI fragment from pNK3 was cloned in the opposite orientation in the same vector and treated as described for pNK11 and 12.

pNK22–28, 30, 31: The restriction fragments shown in Figure 4 were obtained from pNK3 and cloned into the same or compatible restriction sites in pDC1, a plasmid containing the *A. nidulans argB* gene (ARAMAYO, ADAMS and TIMBERLAKE 1989). Each clone was linearized at the junction of pDC1 and *A. nidulans* DNA prior to transformation.

RESULTS

Complementation of the *wA3* mutation: DNA from a pKBY2 cosmid library containing wild-type *A. nidulans* inserts (YELTON, TIMBERLAKE and VAN DEN HONDEL 1985) was used to transform strain NK002 (*pabaA1, yA2; wA3; veA1; trpC801*) to tryptophan-independence. Complementation of the *wA3* mutation in this strain was expected to lead to formation of yellow conidia because of the *yA2* mutation. One of 2950 *trpC*$^+$ transformants produced yellow spores. This strain was colony purified. DNA was isolated, subjected to *in vitro* lambda packaging, and used to transduce *Escherichia coli* HB101 to ampicillin resistance. Three colonies grew and cosmid DNA was isolated from them. No differences were found between the electrophoretic patterns of digests of the three cosmids with four restriction endonucleases. Cosmid DNA from each of the three *E. coli* transductants was used to transform NK002 to tryptophan-independence. With each, >50% of the transformants produced yellow conidia.

Localization of the *wA3* complementing activity: The *wA3*-complementing activity was localized to a 13.8-kb *Eco*RI fragment (Figure 1A) by using individual, gel-isolated fragments from CosNK002 to complement the mutation as described by TIMBERLAKE *et al.* (1985). Subclones of this fragment were tested for their ability to complement *wA3* by cotransformation of NK002 with pTA11, containing the *A. nidulans trpC* gene. Figure 1B shows that an *Xho*I fragment from coordinate positions 6.5–10.5 complemented the mutation. Two other fragments containing this *Xho*I fragment also complemented, whereas flanking fragments did not.

Transcription mapping of the *wA* region: To investigate transcription from the putative *wA* region, pNK3 (Figure 2A), 11, 12, 13, 15 and 19 were used to probe blots of gel-fractionated RNA from conidiating cultures (which contain hyphae, conidiophores and conidia), hyphae, or vegetative cells in which development had been artificially induced by forced expression of *abaA* (MIRABITO, ADAMS and TIMBERLAKE 1989). With the exception of pNK13, the clones

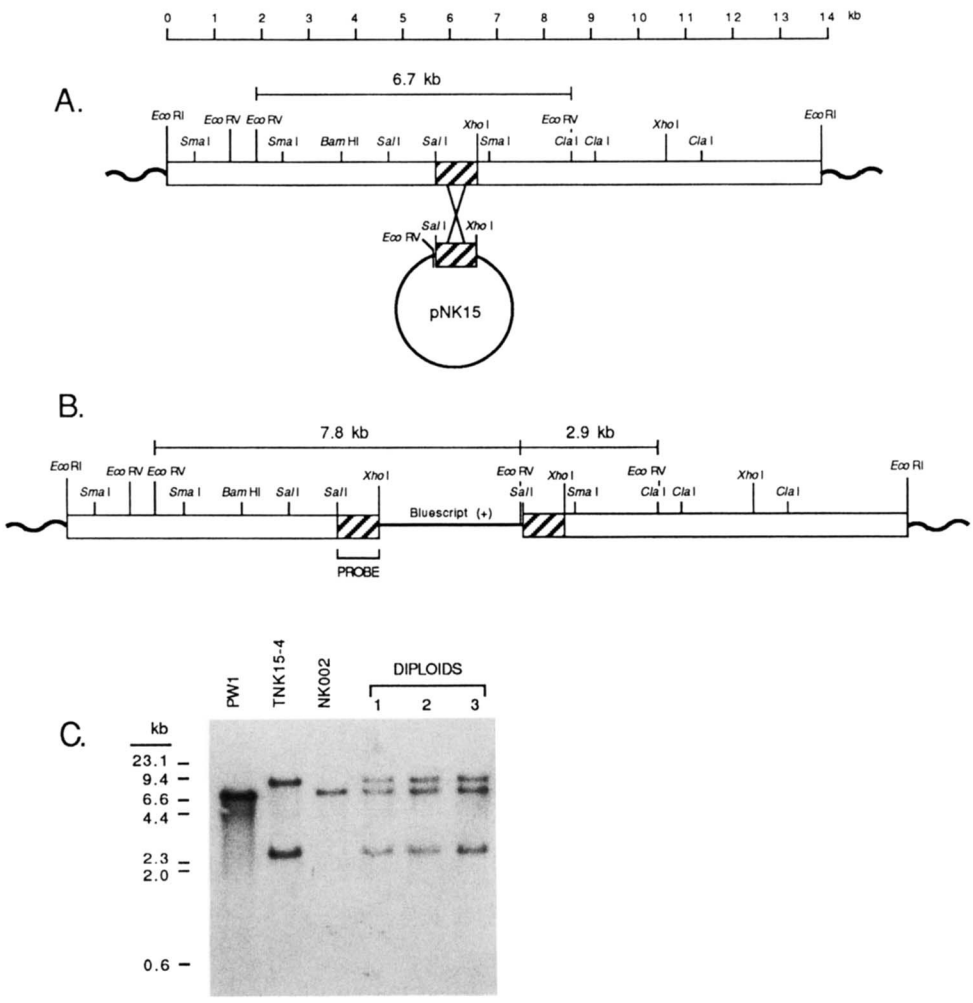


FIGURE 3.—Disruption of the *wA* gene. pNK15 was linearized with *Sal*I, mixed with pSalargB, and the mixture was used to transform the green-spored *A. nidulans* strain PW1 to arginine-independence. Transformants were scored for the production of white spores. Genomic integration of pNK15 by the single crossover event shown in panel A is expected to give rise to a duplication of the hatched *Sal*I-*Xho*I fragment and to the two novel *Eco*RV fragments shown in panel B. (C) DNA from white-spored transformant TNK15-4, PW1, NK002, and three white-spored diploids (1–3) derived from a TNK15-4/NK002 heterokaryon was digested with *Eco*RV and subjected to Southern blot analysis with the 900-bp *Sal*I–*Xho*I fragment (coordinate positions 5.5–6.4) from pNK15 as probe.

hybridized to a 7.5K nucleotide (nt) RNA (Figure 2A) that is absent from hyphae but present in conidiating cultures and in *abaA*-induced cells. Except for clones pNK15 and pNK19, these clones also hybridized to a 2.5K nt RNA that was present in all lanes just below the position of 25S rRNA. A band just above this one and a second band just below the position of 18S rRNA were visible in many blots, but appeared to be artifactual, because they were also present in blots hybridized with unrelated probes and were not present in blots from gels containing poly(A)$^+$ RNA (Figure 5C). These results, together with the complementation data, suggest that the region from the *Bam*HI site at coordinate position 3.7 to the *Xho*I site at coordinate position 10.5 codes for *wA* mRNA as depicted in Figure 2B. The direction of *wA* transcription was determined by blot hybridization with strand-specific RNA probes and is also indicated in Figure 2B. The region from the *Xho*I (10.5) site to the *Eco*RI (13.8) site codes for a 2.5K nt RNA.

Demonstration of *wA* identity: To determine if this transcription unit corresponds to *wA*, we cotransformed *A. nidulans* PW1 (*wA*$^+$; *argB*$^-$) with pNK15, containing a 900-bp *Sal*I–*Xho*I fragment from coordinate positions 5.5–6.4 (Figure 3), and pSal*argB*, containing the *argB* gene. A white-spored, arginine-independent strain (TNK15-4) was selected and colony purified. Southern blot analysis of DNA from PW1 and TNK15-4 showed that pNK15 had integrated by the single homologous recombination event depicted in Figure 3, hence disrupting the putative *wA* transcription unit. Diploids were constructed between TNK15-4 and NK002, and all were white-spored. Southern blot analysis of DNA from the component haploids and several diploids confirmed that the diploids contained *wA* regions from both parents (Figure 3C). In addition we crossed a white-spored disruptant, TNK22-1 (*pabaA1*, *yA2*; *wA::argB*; *ΔargB::trpCΔB*; *veA1*, *trpC801*) with FGSC357 (*biA1*; *wA3*). Of 15,000 progeny scored from recombinant cleistothecia 13 were green spored and 20 were yellow spored giving a recombination frequency of 0.22%. Thus, the insertional mutation is tightly linked to the *wA3* mutation. These results, in conjunction with the complementation and transcription mapping data, confirm that the cloned region contains *wA*.

Disruption analysis of the *wA* region: The limits of the *wA* genetic locus were determined by testing

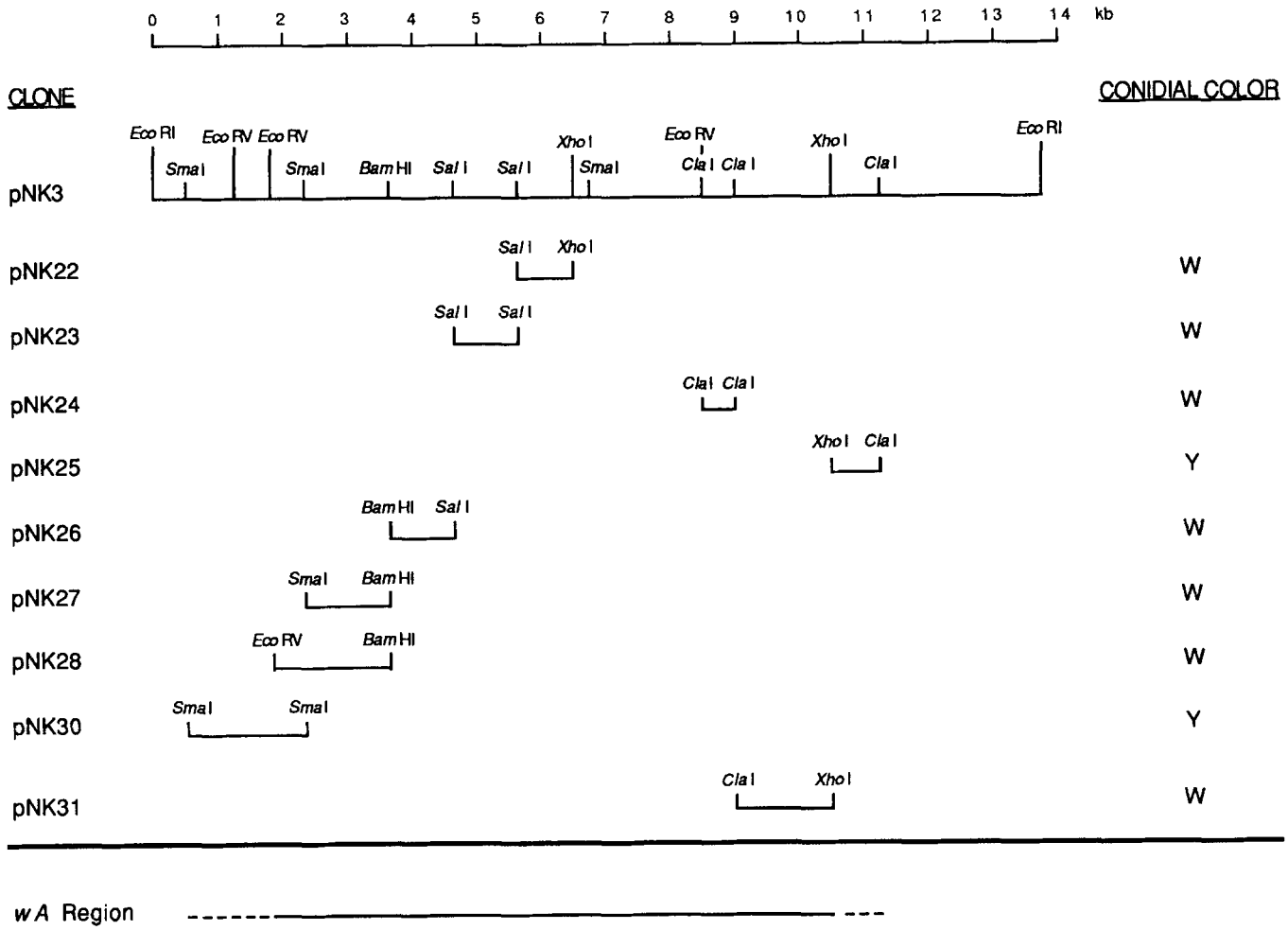


FIGURE 4.—Disruption analysis of the *wA* region. The restriction fragments shown were subcloned into pDC1, containing the *argB* gene. Plasmids were linearized by digestion with restriction enzymes that cut at the junction of pDC1 and the subcloned fragment and used to transform *A. nidulans* strain TMS003 (*pabaA1, yA2*; Δ*argB::trpC*Δ*B*; *veA1, trpC801*) to arginine-independence. Transformants were allowed to conidiate and scored for production of white (W) spores. "Y" indicates that no white-spored colonies were observed in >500 transformants. The inferred position of *wA* is indicated at the bottom of the figure.

the ability of cloned fragments from pNK3 to disrupt gene function by integration events of the type illustrated in Figure 3A. Figure 4 shows that fragments from coordinate positions 1.8–10.5 were capable of disrupting *wA* function and are, therefore, presumably completely contained within the locus. A *Sma*I fragment from coordinate positions 0.5–2.3 produced no white-spored colonies, nor did an *Xho*I-*Cla*I fragment from coordinate positions 10.5–11.1, thereby establishing the outer limits of *wA*.

Developmental regulation of *wA*: To determine the pattern of accumulation of *wA* transcript during conidiophore development, RNA was isolated at various times after inducing development and a gel blot was hybridized with *wA* and *yA* probes. Figure 5 shows that the 7.5k nt *wA* transcript appeared at 15 hr, a time when the first pigmented conidia were being formed, whereas the 2.2K nt *yA* transcript appeared at 10.5 hr, at the time immature conidia were being formed. The *wA* transcript was not detected in poly(A)$^+$ RNA from developmentally abnormal mutant strains carrying the *brlA1*, *abaA1*, *wetA6* or *apsA1* alleles (Figure 5C; see MATERIALS AND METHODS), nor in RNA from purified spores (data not shown). As expected (O'HARA and TIMBERLAKE 1989), the *yA* transcript was not detected in RNA from *brlA1* or *apsA1* strains (Figure 5C).

DISCUSSION

The results presented in this paper show that we have cloned the *A. nidulans wA* gene, because (1) CosNK002 complements the *wA3* mutation at high frequencies, (2) fragments from within the CosNK002 insert also complement the mutation, (3) disruption of the putative *wA* transcription unit through homologous recombination between pNK15 and the genome produces colonies displaying the white-spored phenotype, (4) diploids formed between one such white-spored disruptant strain and a *wA3* mutant strain displayed the *wA*$^-$ mutant phenotype, and (5) a

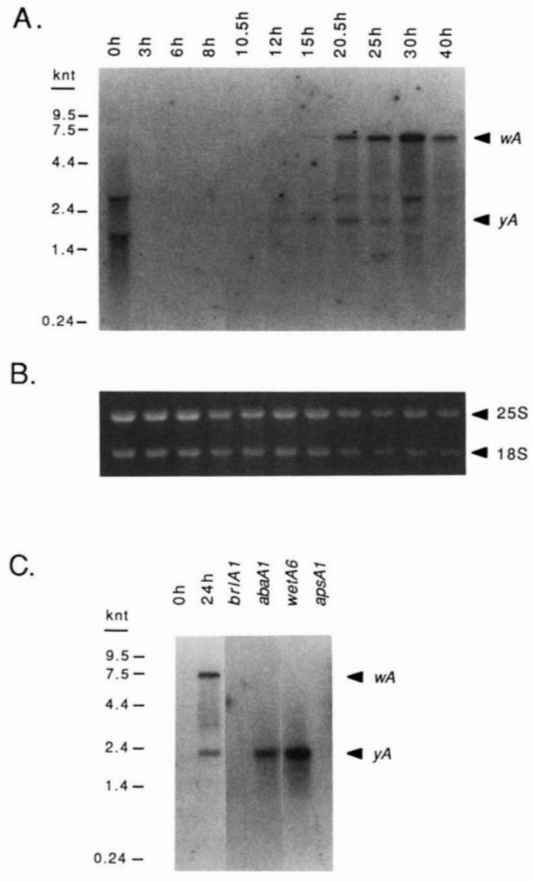


FIGURE 5.—Developmental regulation of *wA*. (A) RNA was isolated from strain FGSC26 at intervals after inducing development. Phialides were first observed at 8 hr postinduction and pigmented conidia were first observed at 15 hr. Gel blots were hybridized with a radiolabeled 900-bp *Sal*I–*Xho*I *wA* internal fragment from pNK15 and a plasmid containing a 1.5-kbp *Bam*HI *yA* internal fragment. (B) An ethidium bromide-stained gel run in parallel with the gel used in panel A. (C) Poly(A)$^+$ RNA, isolated from strain FGSC26 at 0 and 24 hr after inducing development and from strains carrying mutations in the morphogenetic loci *brlA*, *abaA*, *wetA* or *apsA* (see MATERIALS AND METHODS) was hybridized with *wA* and *yA* probes as in blot from panel A.

wA insertional mutation was tightly linked to the *wA3* mutation.

The results further show that the level of *wA* transcript is developmentally regulated. *wA* mRNA was not detected in spores or hyphae, but accumulated in conidiating cultures beginning at the time when conidia first appeared. It also accumulated during artificially induced development in the *alcA*(p)::*abaA* strain TPM1 (Figure 2A; MIRABITO, ADAMS and TIMBERLAKE 1989). Like *yA* mRNA (O'HARA and TIMBERLAKE 1989), *wA* mRNA was not detected in developmentally abnormal, aphialidic strains carrying either the *brlA1* or *apsA1* mutations. In contrast to *yA* mRNA, *wA* mRNA was also not detected in *abaA1* or *wetA6* mutants, both of which produce phialides. Neither *wA* nor *yA* transcripts were detected in mature conidia. Thus, two phialidic strains that either produce no conidia (*abaA1*) or unpigmented conidia that autolyze (*wetA6*) fail to accumulate *wA* mRNA. As *wA* is required for the production of normal conidia and its transcript is absent from spores, it must be expressed in phialides. Thus, our results imply that *abaA* and *wetA* mutations interfere with expression of some phialide-specific genes (*e.g.*, *wA*) without interfering with expression of others (*e.g.*, *yA*) or completely inhibiting phialide formation.

The results of the disruption analyses reported here have some interesting implications concerning the efficiency of plasmid integration by homologous recombination in *A. nidulans*. The recipient strain for the transformation experiments used to map *wA*, TMS003, was deleted for the *argB* locus, therefore precluding integration of the *argB*-bearing transformation plasmids at the corresponding locus by homologous recombination. When the transforming plasmids were linearized with restriction enzymes that cut at the junction of *A. nidulans* DNA and vector sequences, integration at *wA* occurred in >50% of the transformants, whereas with circular plasmids homologous integration at *wA* occurred in <5% of the transformants. Transformations with *argB*-bearing plasmids were also done in *A. nidulans* strain PW1 (*argB*$^-$). Homologous integration at *wA* was also more efficient with linearized plasmids but less frequent (~5%) than with the *argB* deletion strain. These results indicate that integration of plasmids in *A. nidulans* can be directed to specific chromosomal locations by introducing double-strand breaks in the DNA molecules used for transformation, as in *Saccharomyces cerevisiae* (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981).

wA codes for an unusually large polyadenylated transcript (7.5K nt) that, unexpectedly, accumulates later during development than does the *yA* transcript. *wA* transcript accumulation requires *brlA*, *abaA* and *wetA* activities, whereas *yA* requires only *brlA* activity. These differences suggest that even though both genes appear to have related functions and their transcripts may be expressed in the same cell type (phialides), they could be regulated by different mechanisms. The epistatic relationship between *wA* and *yA*, and the observation that *yA* encodes a *p*-diphenol oxidase present in spore walls, has led to the hypothesis that *wA* encodes an enzyme responsible for synthesis of a yellow pigment intermediate that is converted to the mature green form by the *yA* product (PONTECORVO *et al.* 1953; CLUTTERBUCK 1972; LAW and TIMBERLAKE 1980; KURTZ and CHAMPE 1982). The fact that cell walls of *wA*-mutant conidia lack some wall components (OLIVER 1972; CLAVERIE-MARTIN, DIAZ-TORRES and GEOGHEGAN 1988) suggests a more complex function for *wA* than pigment intermediate synthesis. DNA sequence analysis and *in situ* localization of the *wA* product will help in elucidating

the function of *wA* in spore differentiation.

This work was supported by U. S. Public Health Service grant GM37886 to W.E.T. We wish to thank JEAN BOUVIER for his valuable assistance in defining the limits of the *wA* locus. We also thank KATHY SPINDLER, CLAIBORNE GLOVER, SUE WESSLER and SIDNEY KUSHNER for their critical reviews of the manuscript, and our colleagues in the laboratory, especially PETE MIRABITO and TOM ADAMS, for their helpful suggestions and discussions during the course of this work. While this work was in progress TILBURN, ROUSSEL and SCAZZOCCHIO (1990) cloned *wA* by a different route. We thank them for communicating unpublished results and sharing their *wA*-containing clone.

LITERATURE CITED

ARAMAYO, R., T. H. ADAMS and W. E. TIMBERLAKE, 1989 A large cluster of highly expressed genes is dispensable for growth and development in *Aspergillus nidulans*. Genetics **122:** 65–71.

AUSUBEL, F. M., R. BRENT, R. E. KINGSTON, D. D. MOORE, J. A. SMITH, J. G. SEIDMAN and K. STRUHL, 1987 *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

CLAVERIE-MARTIN, F., M. R. DIAZ-TORRES and M. J. GEOGHEGAN, 1988 Chemical composition and ultrastructure of wild-type and white mutant *Aspergillus nidulans* conidial walls. Curr. Microbiol. **16:** 281–287.

CLUTTERBUCK, A. J., 1972 Absence of laccase from yellow-spored mutants of *Aspergillus nidulans*. J. Gen. Microbiol. **70:** 423–435.

CLUTTERBUCK, A. J., 1974 *Aspergillus nidulans*, pp. 447–510 in *Handbook of Genetics*, Vol. 1, edited by R. C. KING. Plenum, New York.

KÄFER, E., 1977 Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv. Genet. **19:** 33–131.

KURTZ, M. B., and S. P. CHAMPE, 1982 Purification and characterization of the conidial laccase of *Aspergillus nidulans*. J. Bacteriol. **151:** 1338–1345.

LAW, D. J., and W. E. TIMBERLAKE, 1980 Developmental regulation of laccase levels in *Aspergillus nidulans*. J. Bacteriol. **144:** 509–517.

MARSH, J. L., M. ERFLE and E. J. WYKES, 1984 The pIC plasmid and phage vectors with versatile cloning sites for recombination selection by insertional inactivation. Gene **32:** 481–485.

MIRABITO, P. M., T. H. ADAMS and W. E. TIMBERLAKE, 1989 Interactions of three sequentially expressed genes control temporal and spatial specificity in *Aspergillus* development. Cell **57:** 859–868.

O'HARA, E. B., and W. E. TIMBERLAKE, 1989 Molecular characterization of the *Aspergillus nidulans yA* locus. Genetics **121:** 249–254.

OLIVER, P. T. P., 1972 Conidiophore and spore development in *Aspergillus nidulans*. J. Gen. Microbiol. **73:** 45–54.

ORR-WEAVER, T. L., J. W. SZOSTAK and R. J. ROTHSTEIN, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:** 6354–6358.

PONTECORVO, G., J. A. ROPER, L. M. HEMMONS, K. D. MACDONALD and W. J. BUFTON, 1953 The genetics of *Aspergillus nidulans*. Adv. Genet. **5:** 141–238.

TILBURN, J., F. ROUSSEL and C. SCAZZOCCHIO, 1990 Insertional inactivation and cloning of the *wA* gene of *Aspergillus nidulans*. Genetics **126:** 81–90.

TIMBERLAKE, W. E., 1986 Isolation of stage- and cell-specific genes from fungi, pp. 343–357 in *Biology and Molecular Biology of Plant-Pathogen Interactions* (NATO ASI Series, Vol. H1), edited by J. BAILEY. Springer-Verlag, Berlin.

TIMBERLAKE, W. E., M. T. BOYLAN, M. B. COOLEY, P. M. MIRABITO, E. B. O'HARA and C. E. WILLETT, 1985 Rapid identification of mutation-complementing restriction fragments from *Aspergillus nidulans* cosmids. Exp. Mycol. **9:** 351–355.

WRIGHT, P. J., and J. A. PATEMAN, 1970 Ultraviolet-sensitive mutants of *Aspergillus nidulans*. Mutat. Res. **9:** 579–587.

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.

YELTON, M. M., W. E. TIMBERLAKE and C. A. M. J. J. VAN DEN HONDEL, 1985 A cosmid for selecting genes by complementation in *Aspergillus nidulans*: selection of the developmentally regulated *yA* locus. Proc. Natl. Acad. Sci. USA **82:** 834–838.

Communicating editor: R. L. METZENBERG