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A Pcl-Like Cyclin of *Aspergillus nidulans* Is Transcriptionally Activated by Developmental Regulators and Is Involved in Sporulation

NIKLAS SCHIER, RALF LIESE, AND REINHARD FISCHER*

Laboratorium für Mikrobiologie, Philipps-Universität Marburg and Max-Planck-Institut für Terrestrische Mikrobiologie, D-35043 Marburg, Germany

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The filamentous fungus *Aspergillus nidulans* reproduces asexually through the formation of spores on a multicellular aerial structure, called a conidiophore. A key regulator of asexual development is the TFIIIA-type zinc finger containing transcriptional activator Bristle (BRLA). Besides BRLA, the transcription factor ABAA, which is located downstream of BRLA in the developmental regulation cascade, is necessary to direct later gene expression during sporulation. We isolated a new developmental mutant and identified a leaky *brlA* mutation and the mutated *Saccharomyces cerevisiae* cyclin homologue *pclA*, both contributing to the developmental phenotype of the mutant. *pclA* was found to be 10-fold transcriptionally upregulated during conidiation, and a *pclA* deletion strain was reduced three- to fivefold in production of conidia. Expression of *pclA* was strongly induced by ectopic expression of *brlA* or *abaA* under conidiation-suppressing conditions, indicating a direct role for *brlA* and *abaA* in *pclA* regulation. PCLA is homologous to yeast Pcl cyclins, which interact with the Pho85 cyclin-dependent kinase. Although interaction with a PSTAIRE kinase was shown in vivo, PCLA function during sporulation was independent of the *A. nidulans* Pho85 homologue PHOA. Besides the developmental regulation, *pclA* expression was cell cycle dependent with peak transcript levels in S phase. Our findings suggest a role for PCLA in mediating cell cycle events during late stages of sporulation.

Asexual sporulation is a widely distributed reproductive mode among fungi. For most species of agricultural or medical importance, spores serve as the means of distribution and infection. The genetic mechanisms of sporulation of *Aspergillus nidulans* and *Neurospora crassa* have been studied in detail (2, 16). The life cycle of *A. nidulans* consists of two developmental phases, a sexual phase and an asexual one, which are both triggered by environmental and endogenous signals. Asexual development begins with the extension of an aerial hypha, the conidiophore stalk, from a specialized thick-walled foot. After apical extension, the stalk tip begins to swell and forms the vesicle, from which two layers of cells are produced by synchronous budding. The first cell generation, called metulae, buds two or three times to produce the second cell generation, the sporogenic phialides. Repeated asymmetric divisions of the phialides lead to long chains of green-pigmented, mitotically derived spores, called conidia. The molecular genetic analysis of asexual sporulation of *A. nidulans* revealed that several hundred genes are differentially expressed during the formation of the conidiophore (2, 27, 46, 47).

Analysis of aconidial mutants and mutants that develop aberrant conidiophores revealed genetic interactions directing conidiophore formation (3, 12, 27). Initiation of asexual development was shown to be regulated by a group of early genes, termed fluffy genes, due to the cotton-like appearance of mutant colonies (23, 52). Later expression of conidiation-specific genes was shown to be mainly regulated by three developmental genes, *brlA*, *abaA*, and *wetA*. These latter genes were proposed to define a central, linear regulatory pathway responsible for proper temporal and spatial gene expression during conidiophore development and spore maturation (8, 34). *brlA* encodes a TFIIIA-like zinc finger transcription factor expressed early during conidiophore formation and was shown to be necessary and sufficient for directing sporulation (1). The *brlA* locus consists of two overlapping transcription units, both essential for normal development, although their products seem to have redundant functions (40). *abaA* is activated by *brlA* during the middle stages of conidiophore development. ABAA contains an ATTS DNA binding domain and is, like BRLA, required for transcriptional activation of several sporulation-specific genes (4, 34). *abaA* induces *wetA* expression which, in response, stimulates expression of further structural conidiophore-specific genes. In addition to this central, linear regulation pathway, the modifier genes *medA* and *stuA* were found to be responsible for the correct spatial and temporal expression of *brlA* (9, 32, 33). While *medA* function during development is still unknown in detail, *stuA* was analyzed at the molecular level. It encodes a transcription factor with an APSES DNA-binding motif (15).

In vegetative hyphae of *A. nidulans*, nuclear division is not necessarily coupled to septation, resulting in filamentous growing, multinucleate cells (13). This is different for all cell types of the conidiophore produced from the vesicle where a complex switch of cell and nuclear division and cell growth occurs. Metulae, phialides, and conidia are uninucleate and of determined size and volume, which requires strict coordination of nuclear division and cytokinesis. Metulae undergo limited mitotic divisions to produce two or three phialides, whereas the phialide nucleus divides up to 100 times to form a chain of conidia. Conidia immediately arrest in the G_1 phase of the cell cycle until they are induced upon germination. At the same time as this dramatic change in the cell cycle, a switch from

* Laboratorium für Mikrobiologie, Philipps-Universität Marburg and Max-Planck-Insitut für Terrestrische Mikrobiologie, Karl-von-Frisch-Str., D-35043 Marburg, Germany. Phone: 49-6421-178-330. Fax: 49-6421-178-309. E-mail: FischerR@mailer.uni-marburg.de.

TABLE 1. Strains of *A. nidulans* used in this study

Strain (mutant)	Genotype	Source or reference
FGSC26	*biA1; veA1*	FGSC
GR5	*pyrG89; wA3; pyroA4; veA1*	G. May, Houston, Tex.
RMSO11	*pabaA1 yA2*; Δ*argB*::*trpC* Δ*B; trpC801 veA1*	43
SRF200	*pyrG89*; Δ*argB*::*trpC*Δ*B; pyroA4; veA1*	21
SSNI2 (9/28)	*pyrG89; wA3; pyroA4; brlA43 veA1*, transformed with pRG1	This study
SSNI10 (9/28-D)	*wA3;* Δ*argB*::*trpC* Δ*B; pyroA4; brlA43*::*pclA veA1*	RMSO11 × SSNI2
SSNI13	*wA3*; Δ*argB*::*trpC*Δ*B; pyroA4; brlA43*::*pclA veA1*, transformed with pDC1 (*argB*) and pSNI26 (*pclA*)	This study
SSNI23	*wA3;* Δ*argB*::*trpC*Δ*B; pyroA4; brlA43*::*pclA veA1*, transformed with pDC1 (*argB*) and pTA111 (*brlA*)	This study
RSH94.4	*methG*; Δ*brlA veA1*	51
SSNI29	*methG;* Δ*brlA veA1*, transformed with pSNI40 (*brlA43*)	This study
SSNI30	*pyrG89;* Δ*argB*::*trpC*Δ*B; pyroA4*; Δ*pclA*::*argB veA1*	This study
SWTA	*pyrG89;* Δ*argB*::*trpC*Δ*B; pyroA4; veA1*, transformed with pDC1 (*argB*)	This study
SSNI37	*pyrG89*; Δ*argB*::*trpC*Δ*B; pyroA4*; Δ*pclA*::*argB veA1*, transformed with pSNI67 (*pclA*)	This study
SSNI39 (9/28β)	*pyrG89; wA3*; Δ*argB*::*trpC*Δ*B; pyroA4; brlA43*::*pclA veA1*	SSNI10 × GR5
PhoΔ17	*pyrG89; wA3 phoA1; pyroA4; veA1*	10
HB9	*pyrG89; phoA1; pyroA4*	10
9/28Δpho1	*pyrG89; wA3 phoA1*; Δ*argB::trpC*Δ*B; pyroA4; brlA43*::*pclA veA1*	SSNI39 × PhoΔ17
SSNI44	*pyrG89; phoA1*; Δ*argB*::*trpC*Δ*B; pyroA4;* Δ*pclA*::*argB veA1*	SSNI30 × HB9
SSNI56	*pyrG89;* Δ*argB*::*trpC*Δ*B; pyroA4; veA1*, transformed with pSNI99 (*alcA*::*pclA-HA*)	This study
SSNI57	*pyrG89*; Δ*argB*::*trpC*Δ*B; pyroA4; veA1*, transformed with pSNI99 (*alcA*::*pclA-HA*)	This study
SO12	*yA2; wA3; nimA5; chaA1 riboB2 veA1*	J. Doonan, Norwich, United Kingdom
JG100	*yA2 pabaA1; methG1; bimE7; veA1*	J. Doonan, Norwich, United Kingdom
TTA292	*biA1; alcA(p)*::*brlA; methG1; veA1*	1
TPM1	*biA1; alcA(p)*::*abaA; methG1; veA1*	34

filamentous to a pseudohyphal, budding-like growth pattern occurs.

In *Saccharomyces cerevisiae*, the passage through the Start cell cycle checkpoint leads to morphological changes in the yeast cell that permit polarized growth towards the budding daughter cell. The cell cycle checkpoint which monitors morphogenesis was found to be regulated through the master cell cycle regulator, the cyclin-dependent kinase (CDK) Cdc28 (Cdc2 homologue) (25). Another nonessential CDK, the Pho85 kinase, also appears to be involved in the regulation of morphogenesis. Pho85 has emerged as an important model for the role of CDKs in processes beyond cell cycle control, as it is involved in the regulation of a broad spectrum of cellular processes (reviewed in reference 5) including metabolism, morphogenesis, and transcriptional regulation. The pleiotropic nature of Pho85 function has been ascribed to its association with multiple cyclin partners, of which 10 are known so far. A yeast strain lacking the entire Pcl1/Pcl2 subfamily of Pho85 cyclins displays strong morphological defects such as elongated buds, random budding in diploids, and delocalized actin patches (24, 31, 45). The main regulator of the cell cycle in *A. nidulans* is the Cdc2 homologue $NIMX^{cdc2}$, which is required throughout the cell cycle (38). As in most eukaryotic cells, $NIMX^{cdc2}$ is associated with the B-type cyclin $NIME^{cyclinB}$, which mediates $NIMX^{cdc2}$ activity (38). Thus far, NIME was the only known cyclin in *A. nidulans.* In addition to Cdc2-cyclin B activity, the β-casein kinase NIMA is also required for entry into mitosis (37, 58). NIMA is necessary for the nuclear localization of $NIMX^{cdc2}$-$NIME^{cyclinB}$ complex and was found to induce chromatin condensation, most likely by phosphorylation of histone H3 (14, 53, 58). There is a second CDK known in *A. nidulans* (PHOA) which is homologous to the yeast Pho85 kinase and was shown to control developmental responses to phosphorus-limited growth (10) but appears not to be directly involved in cell cycle regulation or morphogenesis.

Several experimental findings suggest the coupling of cell cycle requirements to the specific morphological requirements of developmental cell types in *A. nidulans*. It was shown that the central regulator of asexual sporulation in *A. nidulans* BRLA activates cell cycle gene expression, such as that of *nimE* and *nimX* (57). Furthermore, a specific *nimX* mutation, which makes NIMX resistant to negative regulation by tyrosine phosphorylation, was found to display a strong defect in conidiophore morphogenesis, although the strain was not impaired in cell cycle progression (55–57). The mutant produced septated conidiophore stalks in contrast to the unseptated wild-type conidiophore stalks and was impaired in the development of the correct cell types of the conidiophore (57).

Here, we describe the isolation and characterization of a second cyclin gene of *A. nidulans*, named *pclA*, which is specifically required for conidiation, suggesting a CDK-PCLA kinase function during sporulation.

MATERIALS AND METHODS

Strains, plasmids and culture conditions. Supplemented minimal and complete media for *A. nidulans* were prepared as previously described, and standard strain construction procedures were used (19). A list of *A. nidulans* strains used in this study is given in Table 1. Standard laboratory *Escherichia coli* strains (XL-1 Blue and Top 10 F′) were used. Plasmids and cosmids are listed in Table 2. To synchronously induce differentiation of conidiophores, a thin mat of mycelia was filtered from liquid culture and placed upon an agar plate. For temperature-sensitive mutant strains, 42°C was considered the restrictive temperature. For the isolation of cell cycle phase-specific RNA, the wild-type strain FGSC26 was blocked at the beginning of S phase by incubation in the presence of 90 mM hydroxyurea for 4 h, which completely blocks nuclear division (7). A *nimA5*- and a *bimE7*-carrying strain were grown for 8 h at a permissive temperature and then blocked at late G_2 (*nimA5*) and M phase (*bimE7*), respectively, through a shift to restrictive temperature for 3 h. Conidium production was determined with confluent plate cultures. A total of 10^6 conidia of a fresh spore solution that had been washed three times in 0.85% NaCl–0.02% Tween 20 solution were inoculated with 4 ml of medium containing 0.6% agar and poured onto a 1.5% agar plate. After incubation at 37°C, the top layer was excised with the end of a disposable 1-ml pipette tip (diameter, 0.8 cm) and transferred to 0.5

TABLE 2. Plasmids used in this study

Cosmid or plasmid	Construction	Source or reference
pBluescript KS(−)	Cloning vector	Stratagene, Heidelberg, Germany
pUC18	Cloning vector	MBI Fermentas, St. Leon-Rot, Germany
pCR2.1-TOPO	TA-cloning vector for cloning of PCR fragments	Invitrogen, NV Leek, The Netherlands
pDC1	*A. nidulans argB* gene in pIC20R	6
pRG1	Plasmid containing the *pyr4* gene from *N. crassa*	50
W23F08	*pclA*-containing cosmid	FGSC
pSNI26	3.1-kb *Sma*I/*Eco*RV *pclA*-containing fragment in pUC18 cloning vector	This study
r5d06a1	cDNA clone containing *pclA*	FGSC
pSNI38	2.7-kb *Xba*I fragment from genomic DNA of SSNI10 in pUC18 cloning vector containing the *brlA43* gene	This study
pTA111	4.5-kb fragment of *brlA* genomic region	T. H. Adams
pSNI40	*brlA43* allele downstream of 2 kb of the natural promoter of *brlA*	This study
pSNI67	3.1-kb *Sma*I/*Hin*dIII *pclA*-containing fragment from pSNI26 and *pyr4* gene in pBluescript KS(−) cloning vector	This study
pSNI35	*pclA* deletion construct; 685 bp of the *pclA* coding region was replaced by the *argB* gene	This study
GTEP1	3xHA-containing plasmid in pBluescript KS(−)	A. P. Mitchell, New York, N.Y.
pSNI39	3.4-kb *Eco*RI *pclA*-containing fragment from W23F08 cloned behind the *alcA* promoter and the *argB* gene cloned into the *Not*I site of pBluescript KS(−)	This study
pSNI99	3xHA in *Eco*47III site of *pclA* (pSNI39)	This study

ml of NaCl-Tween solution. Probes were homogenized with a 5-ml homogenizer (B. Braun Biotech International, Melsungen, Germany), and appropriate dilutions were counted with a hematocytometer.

Molecular techniques. Standard DNA transformation procedures were used for *A. nidulans* (59) and *E. coli* (41). For PCR experiments, standard protocols were applied by using a Capillary Rapid Cycler (Idaho Technology, Idaho Falls, Idaho) for the reaction cycles. The 9/28 mutant (strain SSNI2) was isolated as a conidiation-deficient strain derived from a restriction enzyme-mediated DNA integration (REMI) mutagenesis experiment with the strain GR5. Mutagenesis was performed as described earlier (21). To clone *pclA* by complementation of the sporulation defect, SSNI2 was crossed to RMSO11, and an arginine auxotrophic strain was selected from the progeny. This strain, SSNI10, was cotransformed with an ordered chromosome VIII-specific *A. nidulans* genomic library (kindly provided by R. Prade and J. Arnold, Athens, Ga.) (54) together with the *argB*-containing plasmid pDC1 for selection. For identification of the *pclA*-containing cosmid W23F08, subclasses of the library cosmids were pooled and subsequently tested for complementation of the sporulation defect. A 3.1-kb *Sma*I/*Eco*RV fragment was obtained by subcloning the W23F08 cosmid and testing different subclones for complementation. DNA sequencing was performed with the automatic sequencer ALFexpress (Pharmacia Biotech, Freiburg, Germany) and Cy5-labeled primers or by a commercial sequencing company (MWG Biotech, Ebersberg, Germany). Genomic DNA was extracted from the fungus with the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). RNA was isolated with TRIzol according to the manufacturer's protocol (GibcoBRL Life Technologies, Paisley, Scotland, United Kingdom). DNA and RNA analyses (Southern and Northern hybridizations) were performed as described in reference 41.

Protein extracts, IP, and Western blotting. Overnight cultures of *Aspergillus* cells were harvested by being filtered through Miracloth (Calbiochem-Merck, Darmstadt, Germany), dried by being pressed between paper towels, and immediately frozen in liquid nitrogen. After being extensively ground in liquid nitrogen, cells were resuspended in protein extraction buffer (20 mM Tris-HCl [pH 8], 0.05% Triton X-100) supplemented with complete protease inhibitors (Roche), 5 mM benzamidine, and 2 mM phenylmethylsulfonyl fluoride. Protein extracts were clarified twice by centrifugation in a Heraeus Biofuge 13 at 13,000 rpm at 4°C for 10 min. For immunoprecipitation (IP) experiments, 10 mg of protein was adjusted to 150 mM NaCl and incubated with 10 μl (5 to 7 μg/μl) of monoclonal antibody HA.11 (clone 16B12; Berkeley Antibody Co., Richmond, Calif.) for at least 2 h at 4°C. Fifty microliters of 50% protein G-agarose (Roche) was added, and incubation was continued for at least 3 h. Agarose beads were pelleted by centrifugation and washed five times with protein extraction buffer. Proteins were eluted by being boiled in sodium dodecyl sulfate sample buffer for 5 min. Aliquots were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot analysis. For Western blot analysis, a monoclonal antibody raised against the hemagglutinin (HA) epitope (16B12; see above) or a polyclonal antibody raised against a PSTAIRE-containing synthetic peptide (Anti-PSTAIR; Upstate Biotechnology, Lake Placid, N.Y.) was used. Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech, Freiburg, Germany) was used for Western blotting, and antibody detection was performed according to the manufacturer's protocol.

Electron microscopy. For scanning electron microscopy (SEM), colonies grown on plates were transferred with a piece of agar onto 5% glutaraldehyde for fixation. After several washes with water, the pieces were transferred to ethylene glycol-monoethyl ether and incubated overnight at room temperature. They were then transferred to water-free acetone and critical point dried. The samples were then sputter coated with gold and observed with a Hitachi S-530 SEM.

RESULTS

Isolation and characterization of a new developmental mutant. *A. nidulans* transformants derived from a REMI mutagenesis experiment with *Sma*I as a restriction enzyme were screened for strains with abnormal conidiophore morphology. One of these mutants (9/28), which appears as red-brownish colonies due to the lack of colored conidiospores (Fig. 1, upper panels), was colony purified and crossed to a wild-type strain. Since a forced heterokaryon conidiated like the wild type, the mutation appeared to be recessive. Analysis of progeny colonies derived from the sexual cross revealed a ratio of 1:1 of conidiating to nonconidiating colonies. This suggested that the defect is due to a single mutated locus. Southern blot analysis of several mutant progenies revealed that the integration of the plasmid used for transformation was not linked to the mutant phenotype (data not shown).

The mutation did not affect vegetative growth, hyphal morphology, or branching, and the mutant was able to produce viable ascospores after self-mating as well as after crosses with different strains. The timing of initiation of asexual sporulation and the number of conidiophores were not altered in the mutant compared to the wild type. However, the mutant fails to produce chains of conidiospores and displays abnormal conidiophore morphology (Fig. 1, lower panels). The swollen vesicle at the end of the stalk produces the first cell generation of the conidiophore, the metulae. These cells are of determined size and volume and appear to be correctly developed in the mutant. In contrast to the wild type, however, the mutant fails to produce the layer of sporogenic phialides but develops mul-

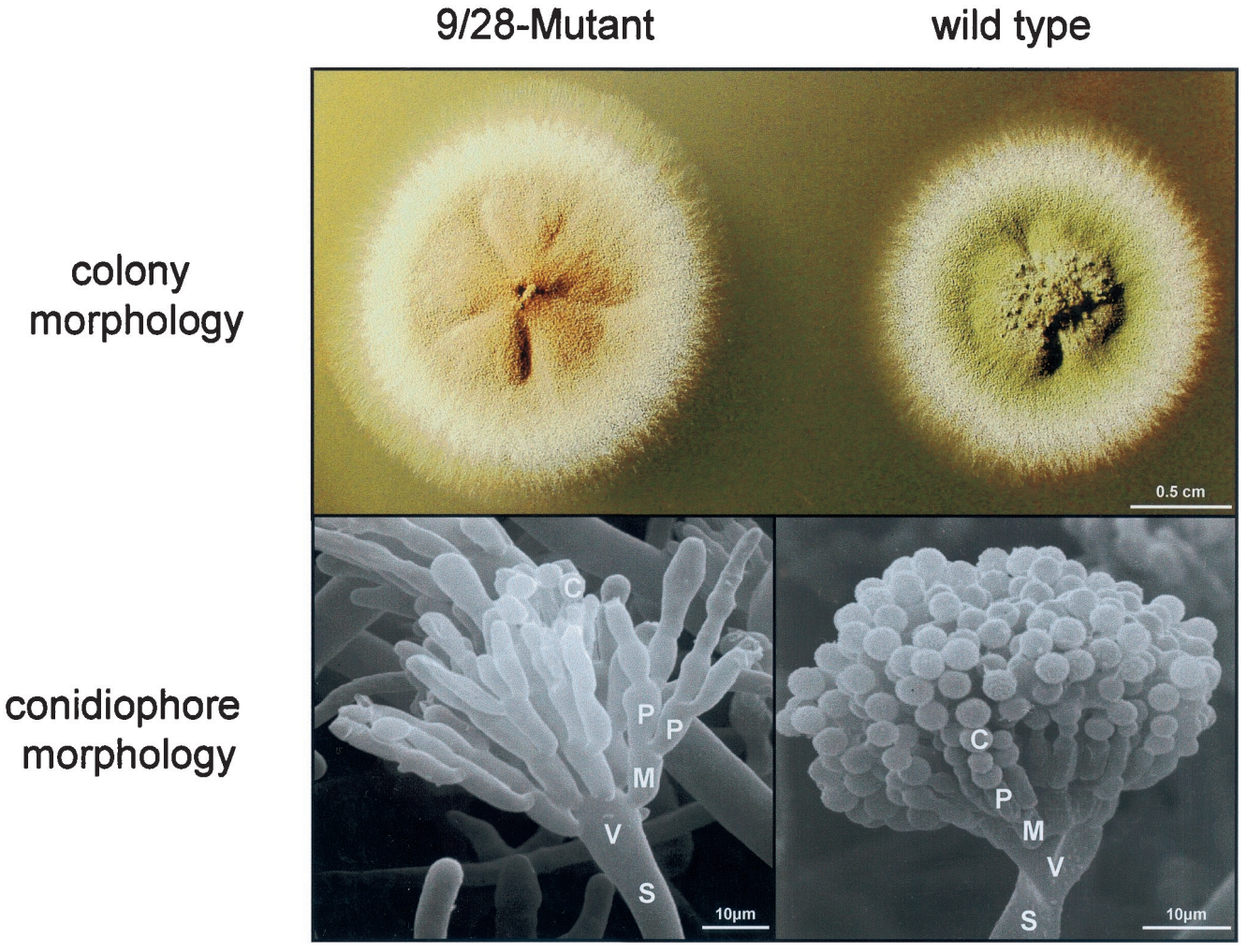

FIG. 1. Phenotype of the 9/28 mutant. (Top) A 9/28 mutant (SSNI2) and a wild-type strain (FGSC26) were grown for 2 days on an agar plate. (Bottom) SEM picture of a 9/28 mutant and a wild-type conidiophore. S, stalk; V, vesicle; M, metula; P, phialide; C, conidium. The wild-type conidiophore picture is reprinted from reference 21 with permission.

tiple generations of phialides instead. Occasionally, single spores are observed at the tip of these cells. Sometimes the cells derived from the metulae are hypha-like elongated structures or resemble reiterated conidiophore stalks (Fig. 1). The phenotype resembles in some way the *abacus* mutant, but a cross of a 9/28 mutant and an *abaA* mutant strain revealed that a different gene was affected in the 9/28 mutant. In this paper, we show that the 9/28 mutant has a composite phenotype due to a tightly linked double mutation. Through recombination during the REMI mutagenesis, the developmental regulator gene *brlA* was fused to the promoter region of the new identified cyclin homologue *pclA* (see below). As a result, both genes were mutated and contribute to the 9/28 developmental phenotype.

Molecular cloning of the *pclA* gene. Since the mutated locus was not tagged to the integration of the transformation plasmid, we isolated the corresponding gene through complementation with an ordered genomic cosmid library of *A. nidulans*. A 9/28 mutant strain carrying an *argB* mutation was constructed (SSNI10) and used as a recipient strain of pooled cosmids from the library cotransformed with the *argB*-containing plasmid pDC1. Transformants were screened for spore production, which could easily be detected upon examining their colony color. Successive transformation of the mutant strain with subclasses of the cosmid library led to the identification of one cosmid (W23F08), which complemented the sporulation defect of the mutant ectopically. Subcloning of this cosmid revealed a 3.1-kb *Sma*I/*Eco*RV fragment, which complemented the sporulation defect of the mutant with high frequency, suggesting that the entire gene was located on this clone (pSNI26) (Fig. 2A). With this fragment as a probe, a 2.2-kb transcript was detected in wild-type RNA (Fig. 2B).

Sequencing of the DNA fragment led to the identification of an open reading frame (ORF) of 420 amino acids (aa), which showed strong homology to yeast cyclins Pcl1, -2, and -9 (Fig. 2C). The *Aspergillus* gene was therefore named *pclA*.

Structure of the *pclA* locus. The 3.1-kb *pclA*-containing restriction fragment and 500 bp of the gene locus upstream and downstream of the *Sma*I and the *Eco*RV restriction site were sequenced. The *pclA* coding region was sequenced on both strands. A corresponding cDNA clone was found in the *A. nidulans* cDNA sequencing project of the Advanced Center for

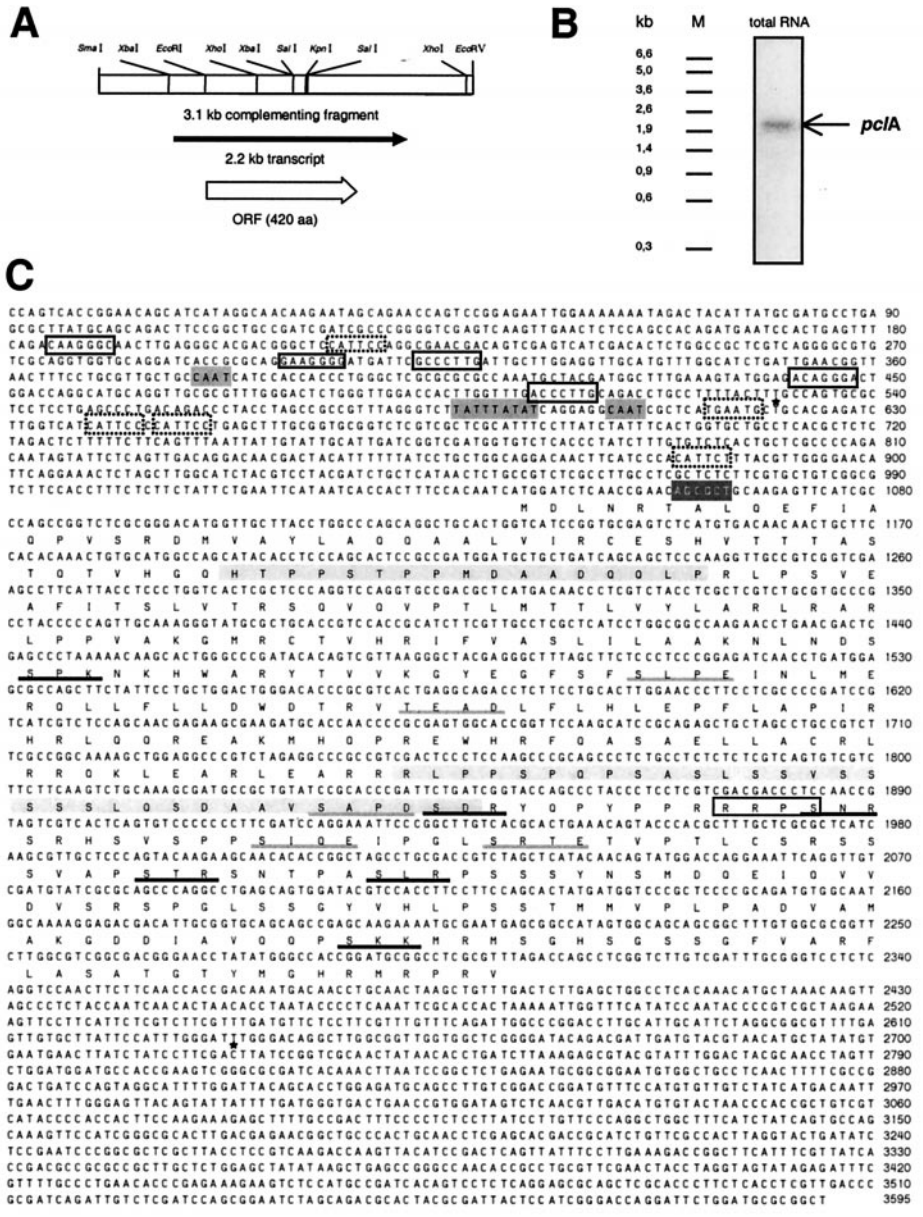

FIG. 2. Molecular analysis of *pclA*. (A) Partial restriction map of the *pclA* locus. The location of the transcript and the ORF is indicated. (B) Detection of the 2.2-kb *pclA* transcript. Fifteen micrograms of total RNA of the wild-type strain FGSC26 was fractionated by denaturing gel electrophoresis and transferred to a nylon membrane. The membrane was hybridized with a gene-specific [α-^{32}P]dATP-labeled probe for *pclA* and exposed to an X-ray film for 2 days. (C) The sequence of the *pclA* locus was determined with the 3.1-kb complementing restriction fragment shown in panel A or the corresponding *pclA* cosmid as a template and synthetic oligonucleotides as primer. The coding region was sequenced on both strands. The predicted transcriptional start site (http://www.fruitfly.org) and the end of a cDNA obtained from the Advanced Center for Genome Technology (University of Oklahoma) are marked above the sequence (*). TATAAA- and CAAT-like boxes in the promoter region are marked in dark grey. Putative binding sites of the transcription factors BRLA (in open boxes) and ABAA (in dotted boxes) are indicated. The derived amino acid sequence of the ORF is given below the sequence in the one-letter code. In the amino acid sequence, the following putative sites are highlighted: PEST sequences (grey), cyclic AMP-dependent protein kinase phosphorylation site (open box), protein kinase C phosphorylation sites (black underlined), and casein kinase II phosphorylation sites (grey underlined). The *Eco*47III restriction site in which the HA epitope was inserted is highlighted with white letters. The sequence is available in the EMBL database under the accession numberAJ272133.

Genome Technology at the University of Oklahoma (www.genome.ou.edu/fungal.html) and kindly provided by the Fungal Genetics Stock Center (FGSC) (Kansas City, Kans.). This 2-kb cDNA clone was sequenced on both strands and compared to the genomic sequence. Since no difference was identified, the *pclA* gene does not contain any intron. The length of the cDNA is in good agreement with the 2.2-kb *pclA* transcript detected in a Northern blot (Fig. 2B). The transcriptional start site was predicted to be 423 bp upstream of the *pclA* ORF (http://www.fruitfly.org). Four hundred forty and 663 bp upstream of the ATG two CAAT boxes and 465 bp upstream of the ATG a TATAAA-like box were identified. In addition, we

found five putative Bristle response elements and five putative Abacus response elements, which correspond to binding sites for the development-specific transcription factors BRLA and ABAA (Fig. 2C). Sequence motifs were indicated if they did not differ by more than one nucleotide from the consensus sequence as defined in references 4 and 11.

The start of the coding region of *pclA* could be assigned on the basis of homology of the encoded protein PCLA to yeast cyclins. The ORF encodes a polypeptide of 420 aa with a calculated molecular mass of 47 kDa. A short ORF of 59 aa which may have a role in translational regulation of *pclA* (26) was detected on the cDNA clone 177 bp upstream of the ATG of the long ORF. Sequence analysis of the deduced PCLA protein led to the identification of several motifs. We found two putative PEST sequences, one cyclic AMP-dependent protein kinase, and six protein kinase C and five casein kinase II phosphorylation sites (Fig. 2C), suggesting posttranslational modification of PCLA.

Homology of PCLA to yeast Pho85 cyclins. In a search for PCLA sequence similarity in the databases, we found homology to the Pho85 cyclins of *S. cerevisiae*. Pho85 is a cyclin-dependent kinase, which plays different roles in the life cycle of *S. cerevisiae* in association with different cyclin subunits. For Pho85-cyclin complexes, roles for regulation of the cell cycle (17, 29, 30), regulation of acid phosphatase expression (20), transcriptional regulation of stress response genes (48), regulation of glycogen metabolism (49), and cell morphogenesis (45) are reported. All 10 cyclin partners of Pho85 were grouped into two subfamilies based on phylogenetic analysis (31). Alignment of PCLA with the Pho85 cyclins revealed that PCLA belongs to the Pcl1/Pcl2 subfamily with the strongest homologies to Pcl1 (31% identity) (Fig. 3). PCLA (420 aa), however, is more than 100 aa longer than its nearest homologues Pcl1, -2, and -9 (279, 308, and 304 aa). The homology was distributed throughout the protein but was most evident in the conserved cyclin box region (Fig. 3).

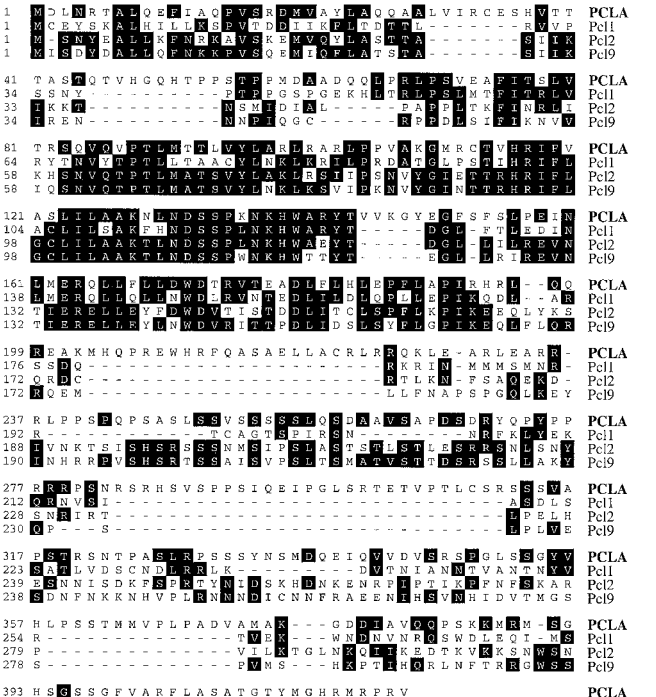

FIG. 3. Alignment of PCLA with yeast cyclins Pcl1, -2, and -9. The alignment was generated with the Megalign program (DNASTAR) by the Jotun Hein method with a PAM250 weighting table. Amino acids which are identical in at least two proteins are highlighted in black.

***pclA* partially complements a double mutant.** After identification of the *pclA* gene we tested whether the mutation leading to the developmental defect was located within the *pclA* gene. Therefore, the *pclA* gene was PCR amplified and cloned from genomic DNA of a mutant strain. Five of the obtained clones were sequenced and compared to the wild-type *pclA* gene. Surprisingly, no differences were detected. To further address the nature of the mutation causing the conidiation defect, total RNA of a mutant strain was isolated and analyzed for the mRNA level of the *pclA* transcript in a Northern blot. In comparison to the 2.2-kb *pclA*-specific transcript of the wild-type strain, a shift of the transcript to 4.5 kb was observed in the mutant. In addition, two weak signals of 2.2 and 2.5 kb appeared (Fig. 4A). From these results we proposed a mutation in the upstream region of *pclA* leading to this altered transcript. Therefore, we analyzed the corresponding genomic region by Southern blotting and found that the restriction pattern of the mutant differs from that of the wild type immediately upstream of a *Sma*I site (data not shown). The 9/28 mutant strain was derived from a REMI mutagenesis experiment where random plasmid integration is favored by the addition of restriction enzyme (in this case, *Sma*I) during the transformation event (22). As mentioned above, the mutant phenotype of the 9/28 mutant was not linked to the plasmid integration, and the recombination in the *pclA* promoter region at the *Sma*I site indicates that this genomic rearrangement is due to the enzymatic activity of *Sma*I during mutagenesis. The shifted transcript would then be a consequence of transcript initiation of an upstream gene running through the *pclA* locus. To isolate this gene, we cloned it from genomic DNA of a mutant strain as a 2.7-kb *Xba*I fragment by colony hybridization, taking the 600-bp *Sma*I/*Xba*I fragment of *pclA* as a probe (Fig. 4B). To our surprise, sequencing of this clone (pSNI38) identified *brlA*, the central regulator of asexual development, as the upstream gene. Furthermore, we found that the *brlA* gene was mutated, as 11 aa of the C terminus are replaced in the mutant by 10 aa translated from the *pclA* upstream region (Fig. 4B). This new *brlA* allele was named *brlA43*. Using the *brlA* gene as a probe in a Northern blot analysis with 9/28 mutant RNA, we found that it hybridized to the same 4.5-kb transcript as the *pclA* gene (results not shown). When we transformed the mutant strain SSNI10 with a *brlA*-containing plasmid, we obtained sporulating transformants with high frequency, indicating that the *brlA43* allele also contributes to the mutant phenotype. However, when we compared the *brlA* transformants (SSNI23) with the transformants obtained with the *pclA* gene (SSNI13), we found that *brlA* rescued the morphological conidiophore defect of the mutant but only partially rescued the sporulation defect, whereas *pclA* rescued the sporulation defect but not the morphology defect (Fig. 5). This indicates a composite phenotype of the 9/28

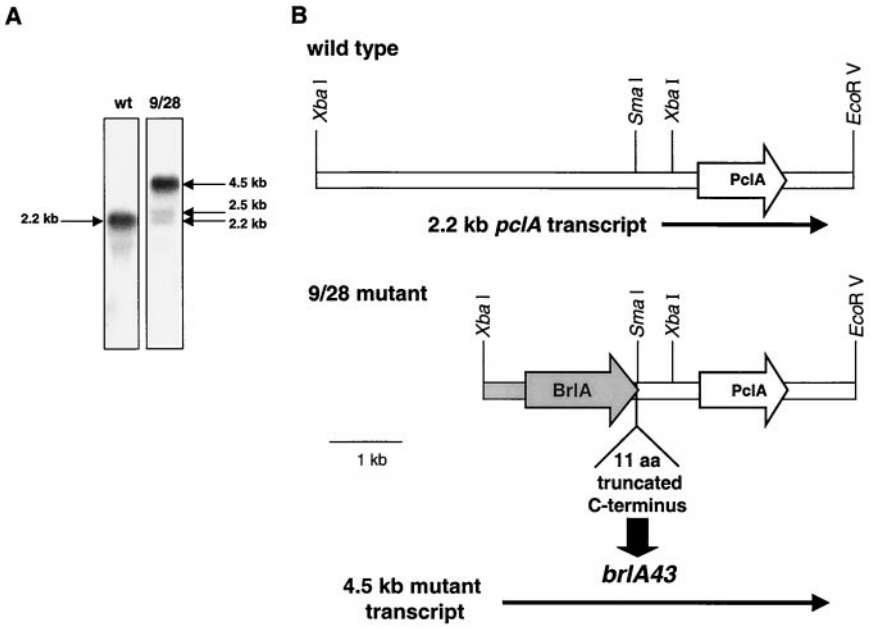

FIG. 4. Determination of the 9/28 mutation. (A) Fifteen micrograms of total RNA of the wild-type strain FGSC26 and a 9/28 mutant strain isolated 25 h after induction of development was analyzed for the expression of the *pclA* transcript on a Northern blot as described in the legend to Fig. 2. (B) Schematic representation of the *pclA* locus in the wild-type strain FGSC26 (top) and a 9/28 mutant strain (bottom) as analyzed by Southern blotting. The locations of the transcripts and the ORFs are indicated. The new identified *brlA* mutant allele was named *brlA43*.

mutant due to a double mutation of the *brlA* and the *pclA* genes.

To prove this, we separated the two mutations independently from the original mutant. Consequently, we cloned the isolated mutant *brlA43* allele downstream of 2 kb of the natural *brlA* promoter and cotransformed this plasmid (pSNI40) into a *brlA* deletion strain (RSH 94.4). Whereas the *brlA* deletion strain only produces stalks, the *brlA43* transformants (SSNI29) displayed the 9/28 conidiophore morphology and a reduction of conidiation (data not shown). In contrast, a *pclA* deletion strain produced conidiophores with wild-type morphology but with a reduced amount of conidiospores (see below).

***pclA* is required for sporulation.** To investigate the role of *pclA* in *A. nidulans* in more detail, we replaced 685 bp encoding 227 aa of the *pclA* gene through the nutritional marker gene *argB* (Fig. 6A). A *pclA*$^+$, arginine auxotrophic strain (SRF200) was transformed with the linearized *pclA* deletion construct to arginine prototrophy. The relatively large flanking regions of the gene locus are necessary to direct the construct to the *pclA* locus through homologous recombination. Among 10 transformants tested in a Southern blot analysis, 2 contained the deletion construct as predicted according to a gene replacement event (Fig. 6B). The *pclA* deletion strains (SSNI30) appeared green-brownish compared to a dark-green wild-type strain, indicating a reduced number of green-pigmented conidiospores (Fig. 7A). The deletion did not affect vegetative growth, initiation of sexual and asexual development, or the number of formed conidiophores. Therefore, sporulation was quantified by plating spores in top agar layers, and conidiospore production was subsequently analyzed as described in Materials and Methods. Wild-type strain SRF200, which was used to construct the *pclA* deletion strain (see above), was transformed with pDC1 to arginine prototrophy, and two different transformants were included in the experiment as wild-type controls to eliminate the effects of media on sporulation (SWTA). As an additional control, a *pclA* deletion strain was retransformed with the entire *pclA* gene, and two transformants were also included in the experiment (SSNI37). The *pclA* deletion strain displayed a conidiation rate of about 30% compared to that of wild-type strains 3 days after inoculation (Fig. 7B). Spore formation was not blocked by *pclA* deletion, as the number of conidia is constantly increasing. Compared to the wild type, however, conidium formation was found to be slowed, and sporulation of the *pclA* deleted strain never reached wild-type rate. After incubation for 5 days, the number of spores in the deletion strain was less than 20% of that of the wild type (data not shown). As the timing of the formation of the conidiophore-specific cell types was as in wild-type strains, the reduction of spore number in the *pclA* deletion strain appears to be caused by slower cell divisions of the sporogenic phialides. The *pclA*-deleted strains which were retransformed with the *pclA* gene behaved like the wild type, proving that the sporulation defect is specific for *pclA* deletion (Fig. 7).

PCLA acts independently of PHOA during sporogenesis. The yeast cyclin homologues of PCLA interact with the CDK Pho85. The *A. nidulans* homologue of Pho85, PHOA, was recently shown to mediate developmental decisions to specific environmental conditions like phosphorus concentration, pH, and inoculation density (10). However, when standard laboratory minimal or complete medium with nonlimiting phosphorus concentrations is used, a *phoA* deletion strain does not display a discernible phenotype. As the *pclA* deletion strain has a specific phenotype under these conditions (see above), it is unlikely that PCLA interacts with PHOA during sporogenesis. The deletion of a CDK normally displays a more pronounced phenotype than deletion of a single cyclin subunit of a CDK

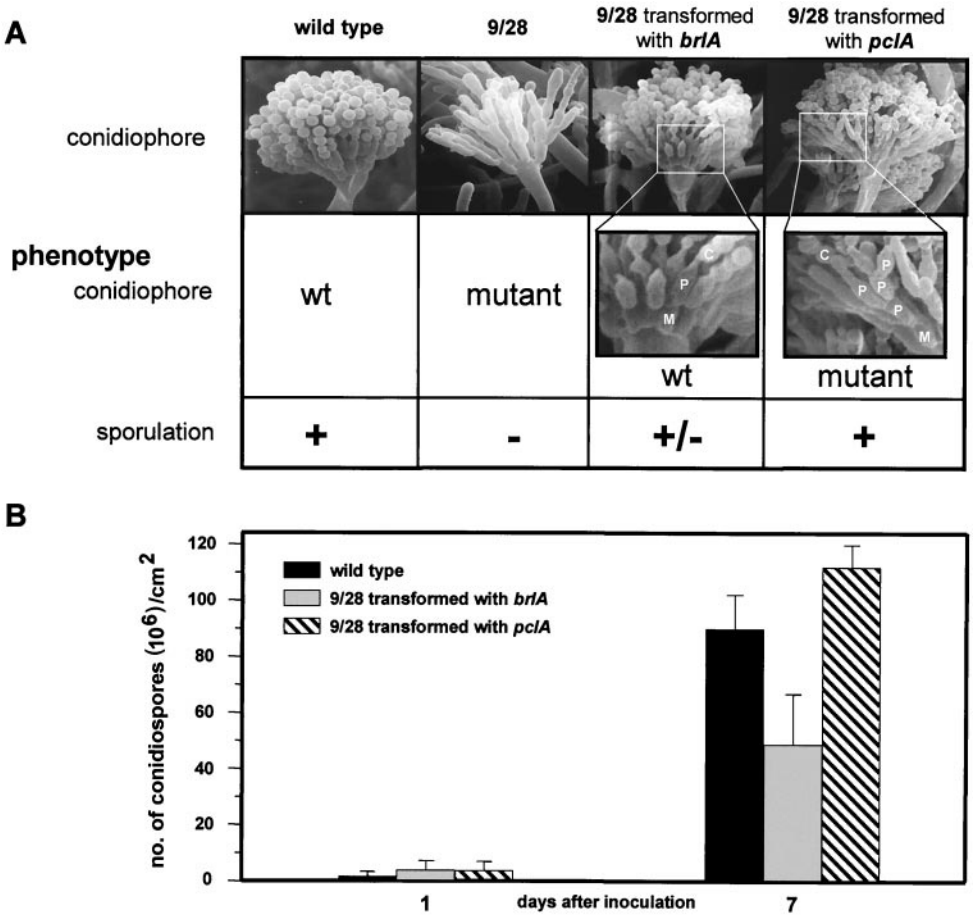

FIG. 5. Transformation of the 9/28 mutant with *brlA* or *pclA*, respectively, only partially complements the mutant phenotypes. The wild-type strain GR5, the mutant strain 9/28-D (SSNI10), and the mutant strain 9/28-D transformed with either the *brlA* (SSNI23) or the *pclA* (SSNI13) gene were phenotypically analyzed. (A) Strains were grown for 2 days on agar plates. (Top) SEM image of the corresponding conidiophores. S, stalk; V, vesicle; M, metula; P, phialide; C, conidium. An image of wild-type conidiophores was taken from reference 21 with permission. (B) Strains were inoculated in a top agar layer, and conidium formation was determined after 7 days as described in Materials and Methods. The spore production of the 9/28 mutant could not be determined as it was under the limit of detection in the experimental setup.

(31). To test that PCLA does not interact with PHOA as a kinase partner during sporogenesis, we constructed a strain carrying the 9/28 mutation and the *phoA* deletion allele *phoA1* by a sexual cross. Progeny strains were isolated and checked for the mutant alleles phenotypically (9/28) and by Southern blot analysis (*phoA*). The progeny strain 9/28ΔphoA1 carrying the original mutation of the 9/28 mutant and the *phoA1* allele was isolated and looked phenotypically like a 9/28 mutant strain. Transformation of this strain with the *pclA* gene led to complementation of the sporulation defect of the strain, indicating yet again that PCLA acts independently from PHOA during sporogenesis (data not shown). Furthermore, we constructed a strain carrying the *pclA* and the *phoA* deletion alleles by crossing the individual mutant strains HB9 (*phoA1*) and SSNI30 (Δ*pclA*). The double mutant progeny strains (SSNI44) also displayed the sporulation defect of the *pclA* deletion strain, demonstrating that the *pclA* mutation is epistatic over *phoA1* (data not shown).

PCLA interacts with a PSTAIRE kinase in vivo. Because the genetic data presented above suggested that PCLA does not require PHOA for its developmental role, we wanted to test whether PCLA interacts with a CDK in vivo. To allow co-IP experiments, we constructed an HA epitope-tagged version (3xHA) of PCLA by cloning the 111-bp epitope carrying DNA into the *Eco*47III site of *pclA.* This restriction site is located at the very N terminus of the encoded protein, outside of the highly conserved cyclin box (see Fig. 2C). To test for functionality of the engineered fusion protein, we transformed a 9/28 mutant strain with the construct and obtained sporulating colonies (data not shown). This demonstrates that the epitope does not interfere with the biological function of the cyclin. However, we were not able to detect the protein in Western blot analyses, probably because the expression level of the protein under the natural promoter is rather low. Therefore we used a construct with the *pclA::HA* gene under the control of the inducible *alcA* promoter (pSNI99). Since the *alcA* promoter is induced by threonine as a carbon source, we grew SRF200 transformants on liquid glucose medium for 14 h, harvested and washed the mycelium, and subsequently incubated it for 4 h in threonine medium. Using protein extracts of these cultures, we detected a specific protein band with a molecular mass of about 55 kDa in a Western blot analysis, which is in good agreement with the predicted mass of 50.6 kDa of the fusion protein (Fig. 8). In addition, several smaller

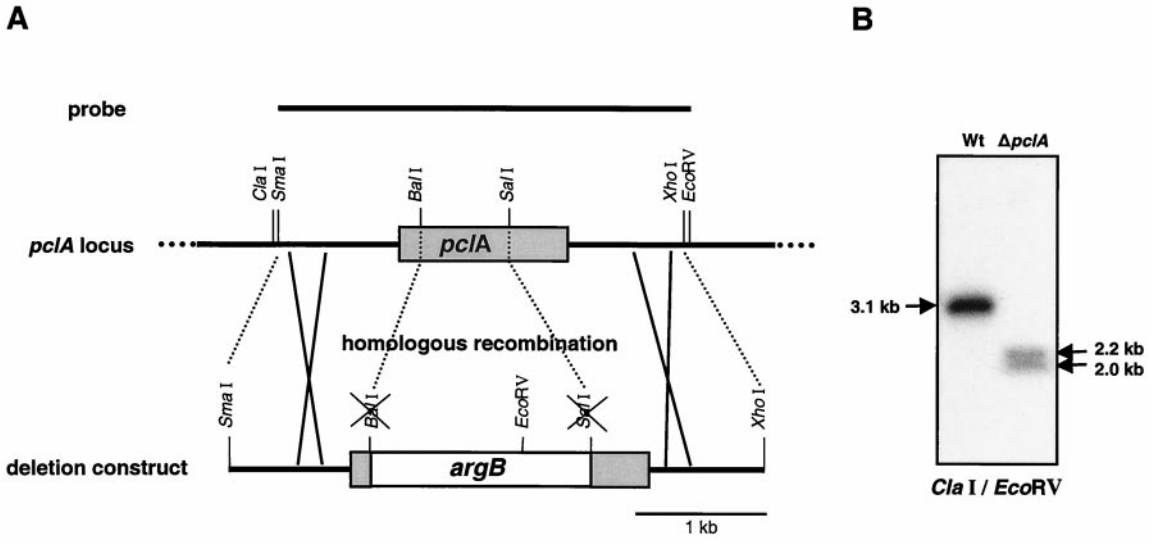

FIG. 6. Deletion of the *pclA* gene. (A) Scheme of *pclA* deletion by homologous recombination. A total of 685 bp between the *Bal*I and the *Sal*I site encoding 227 aa of the *pclA* gene were replaced through the nutritional marker gene *argB* in the deletion construct. A wild-type strain (SRF200) was transformed with the linearized *pclA* deletion construct to arginine prototrophy. The flanking regions of the gene locus are necessary to direct the construct to the *pclA* locus through homologous recombination. (B) Genomic DNA of the wild-type strain SRF200 and the *pclA* deletion strain SSNI30 was digested with *Cla*I and *Eco*RV and analyzed by Southern blotting for replacement of the *pclA* gene. The *Sma*I-*Eco*RV *pclA*-containing fragment was taken as a probe.

degradation products were visible. Two independent transformants (SSNI56 and SSNI57), which showed different expression levels probably due to different integration numbers of the construct, were used. In protein extracts derived from cultures grown on glucose, no signal was obtained (under repressing conditions; results not shown). The protein extracts of the induced cultures were subjected to a co-IP experiment. Putative CDKs were detected with an anti-PSTAIRE antibody, which recognizes the conserved PSTAIRE peptide motif of this protein family. In crude cell extracts of wild-type cells, the antibody reacted with two prominent bands of 30- to 40-kDa molecular mass. After precipitation of the PCLA protein with the anti-HA antibody, one of the putative CDKs was found in the precipitate (Fig. 8). The protein was not found in a control strain where PCLA was not epitope tagged. This shows that PCLA interacts with a CDK in vivo and functions as a cyclin in *A. nidulans.*

***pclA* transcript regulation is complex throughout the life cycle of *A. nidulans*.** Pho85 cyclin family members Pcl1, -2, -5, and -9, the yeast cyclins most closely related to *Aspergillus* PCLA, are expressed in a cell cycle-dependent manner (5). To examine *pclA* gene expression in *A. nidulans*, total RNA was isolated from strains blocked at different stages of the cell cycle, as described in Materials and Methods. Fifteen micrograms of total RNA of each stage was analyzed by Northern blotting for the mRNA level of *pclA*. As an internal control, the cell cycle-regulated *nimA* transcript was taken. In cells blocked in S phase, the *nimA* transcript was absent, whereas a specific signal was obtained in G_2 and a weak signal in M phase-blocked cells (Fig. 9). These findings are consistent with the known transcript regulation of *nimA* (39). The *pclA* transcript was detected in cells blocked at the beginning of S phase but was absent in the G_2- or M-phase-blocked cells (Fig. 9). This result indicates that *pclA* is transcriptionally regulated during the cell cycle with a peak in S phase, suggesting a role for *pclA* during early stages of the cell cycle.

Genes involved in conidiophore formation and sporulation are often regulated at a transcriptional level (28, 43). Since the *pclA* gene was found to play an important role during conidium formation, we analyzed *pclA* expression during conidiation. Asexual development was synchronized by transferring a thin mycelial mat filtered from liquid culture to an agar plate (8). This exposure of cells to an air interphase induces development. Total RNA of wild-type strain FGSC26 was isolated at different time points after induction, and total RNA of each stage was analyzed by Northern blotting for the mRNA level of *pclA*. To quantify the intensity of the signals on X-ray film, we used the computer program ImageQuant (Molecular Dynamics, Sunnyvale, Calif.). The experiment with subsequent quantification was repeated three times with 3, 7, and 15 μg of RNA. In all cases, *pclA* was found to be upregulated at least 10-fold after induction of development, peaking in the late phase of conidiophore development. rRNA was included as an internal control (Fig. 9). The upregulation of *pclA* during late stages of conidiation correlates well with the sporulation phenotype of the deletion strain. The complex regulation pattern during development and cell cycle suggests a role for *pclA* in coupling these cellular processes.

Since regulatory proteins potentially cause phenotypes when overexpressed, we tested *pclA* for this, with the *alcA* promoter to drive the expression. Spore germination, hyphal morphology, vegetative growth, and production of conidia were not affected by increased levels of *pclA* (results not shown).

***pclA* regulation is BRLA and ABAA dependent.** The *pclA* promoter contains five putative response elements for binding of ABAA and BRLA (Fig. 2C). To elucidate whether these regulators in fact control *pclA* transcription, we chose a direct approach and analyzed *pclA* expression under conidiation-sup-

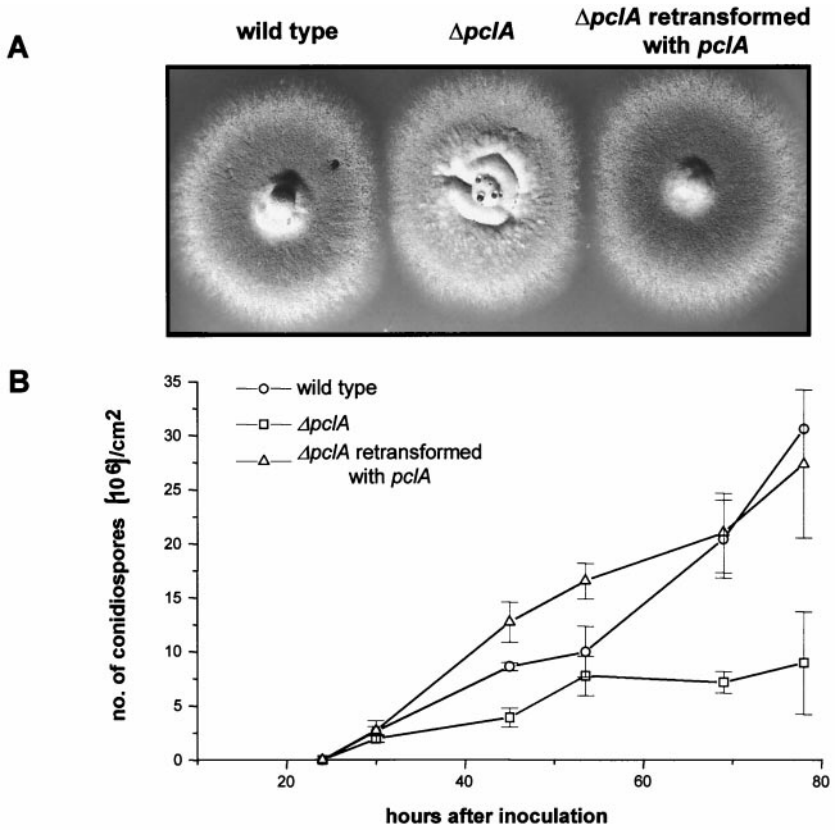

FIG. 7. Dependence of asexual sporulation on PCLA. A wild-type strain (SWTA), a *pclA* deletion strain (SSNI30), and a *pclA* deletion strain retransformed with the *pclA* gene (SSNI37) were analyzed for conidium production. (A) Strains were grown for 2 days on agar plates. (B) Conidiospores were inoculated in a top agar layer, and production of conidia was determined as described in Materials and Methods. Samples were taken at the times indicated.

pressing conditions in liquid culture, using strains carrying the *brlA* or *abaA* gene under the control of the inducible *alcA* promoter. Strains were grown overnight in liquid culture on glucose (repressing) medium before transfer to threonine (inducing) medium. At 0, 1, 2, and 3 h after induction of the *alcA* promoter, samples were taken and analyzed in Northern blot experiments after RNA isolation. As *pclA* transcript is only hardly detectable in vegetative cells, an increase of *pclA* expression after artificial induction of the transcription factors fused to the *alcA* promoter should be readily identifiable. The overexpression of the two regulatory genes after induction was controlled by hybridization of a Northern blot with a corresponding gene-specific probe for either *brlA* or *abaA*. As expected, *brlA* and *abaA* transcripts are absent from vegetative hyphae. Upon transfer to inducing medium, the two regulatory genes were strongly transcribed from the *alcA* promoter (Fig. 10). To investigate whether induced expression of *brlA* or *abaA* in these strains also stimulates *pclA* transcription, we probed duplicate Northern blots with a *pclA*-specific probe. We detected a strong increase of *pclA* transcript after induction of *brlA* as well as *abaA* (Fig. 10). However, in contrast to the full-length *pclA* mRNA (termed *pclA*α) induced upon *brlA* expression, we observed the occurrence of a smaller mRNA species of *pclA* of about 1.7 kb in length (termed *pclA*β) upon *abaA* expression. *pclA*α mRNA was also detectable but not significantly induced under these conditions, whereas the smaller mRNA was found to be strongly induced (Fig. 10, right). This smaller mRNA species is unlikely a product of RNA degradation, as no such shifts were observed in other blots with the same RNA. As we observed the smaller mRNA species only in this artificial system after overexpression of *abaA*, it may be due to recognition of a different transcription initiation site caused by extensive binding of ABAA to the *pclA* promoter. The enhanced expression of *pclA* is not caused by the medium changes, as *pclA* transcript increases only slightly in wild-type cells (Fig. 10, left). We think that this increase is due to *brlA* expression, as we observed a weak *brlA* induction after the shift of wild-type cells to threonine medium (Fig. 10, left column, top panel). Developmental processes are often induced by nutrient limitation. It was reported that *brlA* is induced in liquid culture as a response to carbon or nitrogen limitation (42). Adaptation of the cells to the different carbon source in the induction experiments may cause similar responses leading to the weak *brlA* induction. We also analyzed *stuA* dependence of *pclA* transcription, as we found that *pclA* mRNA amounts persist on a low level in a *stuA1* mutant. In a direct approach with an *alcA*::*stuA*-containing strain as described above, we could not confirm *stuA* dependence of *pclA* transcription (data not shown).

Our results indicate that BRLA and ABAA upregulate *pclA* in collaboration during conidiophore development by directly increasing *pclA* transcription (Fig. 10).

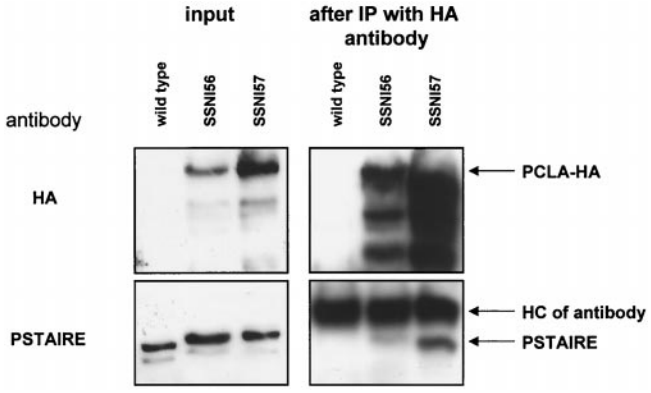

FIG. 8. PCLA interacts in vivo with a PSTAIRE-like kinase. Mycelial extracts were prepared from a wild-type strain (SWTA) and two strains containing *alcA*::*pclA-HA* (SSNI56 and -57) 4 h after *alcA* induction by transfer of an overnight culture from repressing medium (glucose) to threonine medium. (Left) One hundred micrograms of total protein was analyzed by Western blotting with either a monoclonal antibody against the HA epitope (top) or a polyclonal antibody against a PSTAIRE-containing peptide (bottom). (Right) Ten milligrams of total protein was subjected to IP with a monoclonal HA antibody. Aliquots of the IP were analyzed by Western blotting with either a monoclonal antibody against the HA epitope or a polyclonal antibody against a PSTAIRE-containing peptide.

DISCUSSION

The molecular analysis of asexual development in *A. nidulans* led to the identification of a complex regulatory system, which is mainly characterized by transcriptional control mechanisms leading to differential expression of structural genes required for conidiophore formation. As the pattern of cell growth, nuclear division, and cytokinesis changes dramatically during elaboration of the different cell types of the conidiophore, it was proposed that some developmental interactions between the cell cycle and the developmental program must exist (35). Recently, it was shown that the main cell cycle regulators $NIMX^{cdc2}$ and NIMA are upregulated on mRNA and kinase activity levels in a *brlA*-dependent manner (57). Here, we report the isolation of the cyclin *pclA* as a new developmental gene required for the fast, repetitive cell divisions of the phialides, which subsequently lead to long conidium chains of the conidiophore. *pclA* was found to be regulated during the cell cycle and induced through BRLA and ABAA during development. Our data suggest a role for *pclA* in mediating cell cycle events during developmental cell type formation.

pclA was isolated by partial complementation of a new developmental mutant (9/28), later characterized as a double mutant carrying a new leaky *brlA* (*brlA43*) allele fused to the *pclA* locus. The mutant displays a sporulation defect and an altered conidiophore morphology. With the REMI mutagenesis method, applied for the isolation of the 9/28 mutant, a rearrangement of the genome occurred. Surprisingly, this brought the *brlA* gene in close proximity to the *pclA* locus and resulted in a modification of the C terminus of the BRLA protein. A total of 11 aa were replaced through 10 aa. In addition, this mutation caused a readthrough of the *brlA* transcript and a severe reduction of the original *pclA* transcript. Instead, in the wild type we identified two genes upstream of *pclA* with high homology to an endo-β-1,4-glucanase (accession no. AF043595) from *A. aculeatus* and a C-8 sterol isomerase (Swissprot accession no. Q92254) from *N. crassa* (results not shown). Two lines of evidence prove that the BRLA modification is the cause for the morphological conidiophore phenotype of the mutant and that the misfunction or nonfunction mutation of the *pclA* gene due to the fusion transcript causes the sporulation defect of the mutant. (i) Transformation of the mutant with the *brlA* gene could rescue the morphological conidiophore defect of the mutant, but rescue the sporulation defect only partially. Transformation with the *pclA* gene restored the sporulation defect but not the morphology defect. (ii) We were able to separate the two mutations and could show that the corresponding single-mutant phenotypes, established independently from the original mutant, add to the 9/28

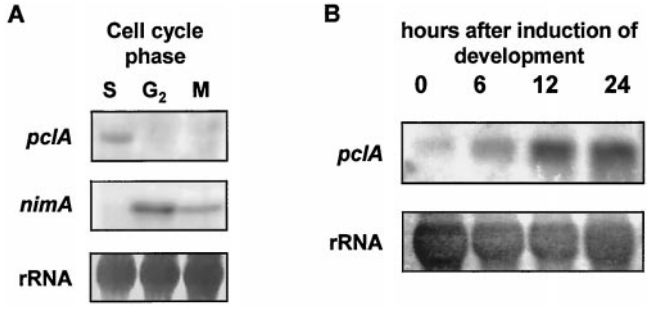

FIG. 9. *pclA* transcription regulation is cell cycle and development dependent. (A) Total RNA of the wild-type strain FGSC26 was isolated 3 h after a hydroxyurea-imposed block in S phase. A *nimA5*- and a *bimE7*-carrying strain were grown for 8 h at permissive temperature (30°C) and then blocked at late G_2 (*nimA5*) and M (*bimE7*) phase through a shift to restrictive temperature (42°C) for 3 h. Total RNA was isolated and analyzed for the mRNA level of *pclA* on a Northern blot as described in Fig. 2. *nimA* was taken as an internal control. (B) Total RNA of the wild-type strain FGSC26 was isolated at different time points after induction of synchronous asexual development by exposure to an air interphase. Fifteen micrograms of total RNA was analyzed for the mRNA levels of *pclA* on a Northern blot as described in Fig. 2. To assure equal loading of the lanes, the RNA on membranes was stained with methylene blue before hybridization.

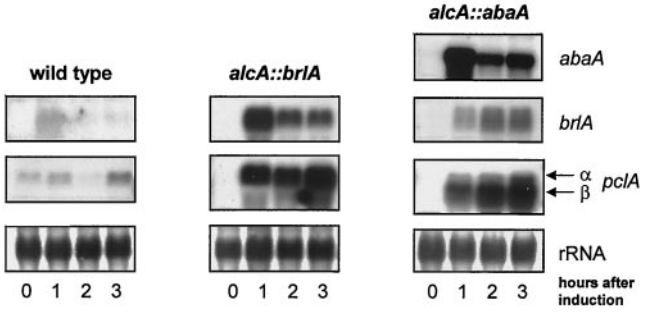

FIG. 10. Induction of *pclA* mRNA by ectopic expression of *brlA* and *abaA*. Total RNA was isolated from a wild-type strain (FGSC26), a strain containing *alcA*::*brlA* (TTA292), and a strain containing *alcA*::*abaA* (TPM1) grown on repression medium (glucose) (0 h) and after *alcA* induction by transfer to threonine medium (1, 2, and 3 h). RNA was analyzed for the mRNA levels of *abaA*, *brlA*, and *pclA*. To assure equal loading of the lanes, the RNA on membranes was stained with methylene blue before hybridization.

double-mutant phenotype. First, transformant strains of a *brlA* deletion strain with the isolated mutant *brlA43* allele displayed the conidiophore morphology of the 9/28 mutant but were reduced in sporulation in comparison to the wild type. Second, deletion of the *pclA* gene leads to a severe reduction of conidiospore production but not to any other morphological changes of the conidiophore. The isolation of a leaky *brlA* allele is in agreement with a recent analysis of different null and leaky *brlA* mutations, where it was shown that the majority of leaky mutations lie in the 3′ half of the gene, possibly in the region that carries the presumptive DNA binding domain (18). Hypomorphic *brlA* alleles permit more extensive development than null mutants, which only form conidiophore stalks but no more differentiated cells, demonstrating the complexity and the central importance of *brlA* in the regulation of sporulation.

We showed that *pclA* is required during sporogenesis by the phialides as a *pclA* deletion strain displays a strong reduction of conidium production. Initiation of development and of all cell types of the conidiophore was not delayed. Spore germination, hyphal morphology, sexual development, or vegetative growth was not affected by *pclA* deletion, suggesting that *pclA* is a new development-specific gene. *pclA* expression was upregulated late during conidiophore formation, which correlates well with the late developmental phenotype of the *pclA* deletion strain. We could induce inappropriate expression of *pclA* by overexpression of the central developmental regulators *brlA* and *abaA* in liquid culture. Since BRLA activates *abaA* and ABAA also induces *brlA* transcription, it could be that the activation of *pclA* is only dependent on one of the transcription factors. However, we believe that both, BRLA and ABAA, contribute to the induction because BRLA overexpression led to an increase of exclusively *pclA*α and ABAA led to a strong induction of *pclA*β and only a weak induction of the α transcript. This makes *pclA* a class A gene as defined in reference 34. Class A genes are regulated by either BRLA or ABAA or both. There are other class A genes known which share with *pclA* not only the expression pattern but also a function late in development, during spore formation. The conidial laccase YA is involved in spore pigment synthesis and the hydrophobine RODA is important for the hydrophobicity of conidia (for a review, see reference 2).

Time course sporulation analysis showed that the speed of spore formation was reduced in a *pclA*-deleted strain compared to the wild type, and even longer incubation of the deletion strain did not result in the same number of spores as in wild-type strains. As mentioned above, this delay was not due to inappropriate initiation of conidiophore formation nor to later differentiation of the conidiophore-specific cell generations, the metulae or phialides, and therefore suggests a slower cell division of the sporogenic phialides leading to this phenotype. All cells of the *A. nidulans* conidiophore derived from the vesicle are uninucleate and are produced in a budding-like fashion, in contrast to the multinucleate, filamentous, vegetative cells. This requires a strict coupling of nuclear division and cell septation and might also be accompanied by an increase of the speed of the cell cycle, given that in a short time up to 100 conidiospores are produced from one phialide. Therefore, *pclA* could be responsible for adaptation of the cell cycle to the fast, repetitive spore formation by the phialides. This likely cell cycle function for *pclA* during development is supported by the expression pattern of *pclA* mRNA in undifferentiated cells. We observed a *pclA* transcript concentration peak in S phase, suggesting a role for *pclA* during early stages of the cell cycle. As no function of *pclA* in vegetative cells could be detected, we think that *pclA* has a role in linking the cell cycle particularly to development during the elaboration of the conidia.

We found that PCLA interacts with a PSTAIRE-like kinase in vivo. Since the yeast PCLA homologues interact with Pho85, it seemed likely that PCLA activates PHOA (Pho85) in *A. nidulans.* PHOA was shown to be required for linking developmental decisions to environmental conditions like pH and phosphorus concentration (10). We could, however, exclude PHOA as a partner for PCLA during sporulation, as a *phoA*-deleted strain did not display the developmental phenotype of a *pclA* deletion strain, suggesting another interacting kinase for PCLA during development. One partner could be a second *phoA*/*PHO85*-related gene in *A. nidulans*, which Bussink and Osmani proposed existed (10). Furthermore, the experimental finding that the main cell cycle CDK $nimX^{cdc2}$ is developmentally regulated in a *brlA*-dependent manner suggests the possibility that PCLA interacts with this kinase. In fission yeast the PCL-like cyclin $PAS1^{+}$ was shown to interact with a Pho85- and a Cdc2-like kinase in vivo; nevertheless, a cellular function could before now only be assigned to Pas1p associated with the Pho85-like kinase Pef1, which appears to be responsible for the activation of G_1-S-specific gene transcription (44).

Besides a function of *pclA* in the regulation of cell cycle events, other roles for *pclA* might also be discussed. Recent analysis of *S. cerevisiae* revealed that Pho85-cyclin complexes (Pcl2 and Pho80) phosphorylate the cell cycle transcription factor Swi5 in vitro (29), suggesting a role for Pho85-cyclin kinase in regulating Swi5 activity. Furthermore, there is experimental evidence that yeast Pcl cyclins link cell cycle decision of the cell to morphogenetic events, probably by modifying the actin cytoskeleton. Pho85-Pcl2 kinase was recently shown to phosphorylate Rvs167p in vitro, which is involved in the organization of the actin cytoskeleton (24). In addition, a diploid strain lacking the entire Pcl1/Pcl2 subfamily displays morphological defects, such as elongated buds, connected chains of cells, and random budding (31, 45). This resembles the formation of the cell types of the conidiophore, which is thought to be a budding-like process. Whether a CDK-PCLA kinase complex regulates transcriptional activation or directs cytoskeletal components specifically required during spore elaboration, however, remains to be elucidated.

So far, only the B-type cyclin NIME and PCLA, described in this paper, were identified experimentally in *A. nidulans* and sequence comparison of yet over 6,000 expressed sequence tags (www.genome.ou.edu/fungal.html) did not reveal the existence of additional cyclins. In contrast to *S. cerevisiae*, where different cyclin subunits associated with the Pho85 or Cdc28 kinase specify its function and activity, the regulation of CDK activities may be different in *A. nidulans*. Whether *Aspergillus* cyclins are able to bind to more than one CDK, which was reported for the novel PCL-like cyclin $PAS1^{+}$ in fission yeast (44) and is known for several cyclins in higher eukaryotes (for a review, see reference 36), and by this means provide the different cellular functions for CDK-cyclin complexes will therefore be of high interest.

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