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Blue light induction of conidiation-specific genes in *Neurospora crassa*

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ABSTRACT

The *con* genes of *Neurospora crassa* are preferentially expressed during a developmental process known as conidiation. We present evidence indicating that transcription of *con-5* and *con-10* is also stimulated by blue light. Transcription of these genes was not photoinducible in *wc-1* and *wc-2* mutant strains. The response of *con-5* and *con-10* to blue light was similar to that of *al-1* and *al-2,* genes involved in carotenoid biosynthesis, and *bli-3* and *bli-4*, blue light inducible genes.

INTRODUCTION

During the asexual phase of its life cycle the ascomycete *Neurospora crassa* proceeds through a developmental process known as conidiation. During conidiation the fungus produces asexual spores, called conidia, on specialized structures known as conidiophores. Several events involved in this complex differentiation process (1) are influenced by the environmental factor, light. Conidiophores develop towards light (2–3). Illuminated cultures produce conidia faster (3–4), and in greater numbers (5), than dark grown cultures. Finally, the circadian rhythm of conidiation is influenced by light (6–8).

A number of genes have been isolated from *Neurospora crassa* with the aim of studying its developmental programs, and the influence of environmental factors on these programs, at the molecular level.Three sets of genes have been cloned that are of potential interest with respect to light-influenced development. First, several genes of unknown function (*con* genes) have been isolated that are expressed more actively during the process of conidiation (9). Second, transcript amounts of two genes, *al-1* and *al-2*, that encode enzymes essential for carotenoid biosynthesis, increase in response to conidiation (Schmidhauser, Sachs, Yanofsky, unpublished results). In addition to this stage specific developmental regulation, transcription of *al-1* (10) and *al-2* (Lauter, Schmidhauser, Yanofsky, Russo, unpublished data) is stimulated by the environmental stimulus blue light. Finally, four additional blue light inducible genes (*bli* genes) have been isolated (11). The functions of the *bli* genes and their expression pattern during asexual differentiation is unknown.

Blue light has a dramatic effect on gene expression in *Neurospora crassa*. It has been estimated that 3–4% (60–80 genes) of all genes expressed during vegetative growth are photoinducible within 60 min following exposure to light (12). mRNA levels for the fast photoreacting genes increase within 2 to 5 min while those of the slower reacting genes increase within 45 min (11).

An aproach to study the mechanism of blue light regulated gene expression in *Neurospora crassa* will be to compare upstream regions of different photoinducible genes, identify homologous sequences and study their role. Towards this goal, we searched for additional light-regulated genes. Since conidiation is influenced by light, and since *con* gene expression varies during conidiation, and other genes (*al-1* and *al-2*) in which such changes occur are known to respond to light, *con* genes appeared to be likely candidates for studies on photoinducibility. Accordingly, we have analyzed the effect of blue light on the mRNA levels of *con* genes.

MATERIAL AND METHODS

Strains

Neurospora crassa wild type strain (St. Lawrence, STa) was provided by J.R.S Fincham (Cambridge University, UK). Strains FGSC 4398 (*wc-1*a), FGSC 4396 (*wc-1*a), FGSC 4408 (*wc-2*a) and R251 (*wc-2*a) were used as sources of *wc* RNA. The *wc* strains are isogenic with the WT (STa) (13). The *wc-2* strain R251 was obtained by UV mutagenesis in the Russo laboratory, is isogenic to STa and is not present in the Fungal Genetics Stock Center (FGSC). The pCon, *con* and *tub-2* clones (9, 19–21, 28) were provided by Charles Yanofsky (Stanford University).

Medium

Modified Vogel's medium (8) was supplemented with 2% (wt/vol) sucrose as a carbon source.

Light induction

Neurospora crassa mycelia were grown in the dark in 75 ml liquid medium in 250 ml flasks to late logarithmical phase and were harvested by filtration in the dark as described (14). Harvested

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mycelial pads were cut in half; one half was photoinduced with blue light (14, 15), and the other half served as a dark control. Blue light was obtained by filtering with two blue light filters (2 mm thick lucite, each; Roehm GmbH, Darmstadt, Germany, symmetrical transmission peak at 450 nm, 0.1% transmission at 360 and 580 nm, and one 4 mm filter BG28 from Jenaer Glaswerk Schott & Gen, Mainz, Germany). It was contaminated neither by UV below 300 nm nor IR light (15). The fluence rate of the blue light was 14 W/m^2. Illumination was for a maximum of 10 min. Mycelia illuminated for 2, 5 and 10 min were immediately frozen in liquid nitrogen. For the 20 min, 30 min and 60 min time points, mycelia were incubated in the dark after 10 min of illumination and prior to freezing. RNA was extracted from these differently treated mycelial segments (16) and hybridized to linearized pCon plasmid DNA in dot blot experiments (17).

Illumination with white light (14) was for 30 min or 60 min. Mycelia illuminated for 30 min or 60 min were immediately frozen in liquid nitrogen. The fluence rate of the blue part of the white light was 6 W/m^2.

Illumination with red light (15) was for 30 min. Red light for photoinduction was obtained by filtering with four lucite sheets (2 mm thick, each; No. 501 from Roehm GmbH, broad transmission peak ranging from 600 to 800 nm, 0.1% transmission at 560 nm). The fluence rate of the red light was 27 W/m^2. Mycelia illuminated for 30 min were immediately frozen in liquid nitrogen.

RNA analysis

For the RNA extraction procedure used see (16). For the RNA dot blot procedure used see (17). For Northern analysis procedure used see (9). For radiolabeling of DNA fragments see (18). For quantification of RNA expression see (11). RNA expression of *con* genes in separate RNA preparations was normalized against the nonphotoinducible clone *n-6* (11).

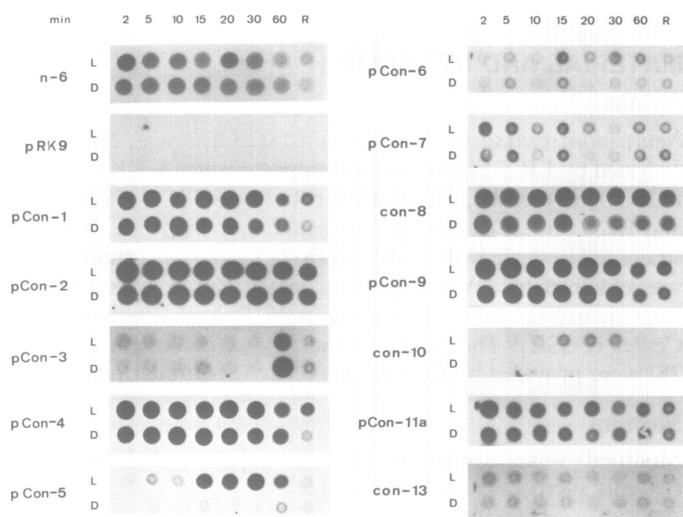

Fig. 1. Kinetics of *con* mRNA synthesis after blue light induction. Mycelia for RNA isolation were irradiated with blue light for a maximum of 10 min. RNA was extracted at different times after the beginning of illumination. Then, 3 μg of total RNA was immobilized per dot and probed with radiolabeled pCon, *con* and *n-6* DNA. Radiolabeled vector (pRK9) was used as a control (27). The extent of hybridization was visualized by autoradiography. Symbols: L, blue light; D, dark; R, 30 min red light illumination. Time is shown in minutes after initiation of illumination.

RESULTS

The first question asked in this study was whether *con* gene expression shared an important regulatory feature with two *al* genes, *al-1* and *al-2*, namely to be under both developmental control and light control. The *con* genes have been isolated as a series of *Echerichia coli* plasmid clones (the pCon series); each plasmid contains one or more genes encoding mRNA species found predominantly or exclusively during conidiation (9). The pCon plasmids contain *Neurospora crassa* genomic DNA inserts of an average length of 5000 bp. The pCon DNAs hybridize to one or more mRNA species (9). The gene (or genes) in these plasmids (pCon-1 − pCon-11a) have been named *con-1, con-2 ... con-n*. The gene corresponding to the most abundant mRNA transcript detected by each plasmid (pCon) that is conidiation-inducible has been given the same number as the plasmid (19). Three *con* genes have been sequenced and characterized further, *con-8* (19), *con-10* (20) and *con-13* (21).

We used pCon-1, pCon-2, pCon-3, pCon-4, pCon-5, pCon-6, pCon-7, pCon-9 and pCon11a plasmid DNA (9) in hybridization experiments to determine if blue light regulated genes are located on these plasmids. To assay blue light regulation, RNA was extracted from mycelial segments that were subjected to different light regimes as described in Material and Methods. Those RNAs were hybridized to linearized pCon plasmid DNA in dot blot experiments (17). Results are shown in Fig. 1. This analysis was also performed with isolated genes *con-8* (19), *con-10* (20) and *con-13* (21) (Fig. 1).

With the exception of pCon-5 none of pCon plasmid DNAs hybridized to mRNA species that were more abundant in

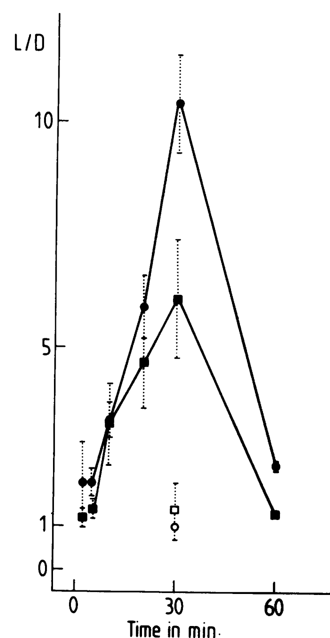

Fig. 2. Kinetics of pCon-5 and *con-10* RNA accumulation following blue light induction. RNA dot blot data are plotted. Autoradiographs obtained from dot blot experiments were analyzed with a 2D LKB Laser Densitometer 2222−010 Ultrascan XL and LKB software. Light / dark values (L/D) are the ratio of the amount of pCon-5 or *con-10* mRNA in blue light-treated versus dark grown mycelia. Each point is the average of two to five RNA dot blots with independent total RNA preparations. Symbols: ●, pCon-5 (blue light); ○; pCon-5 (red light); ■ *con-10* (blue light); □ *con-10* (red light). Time is shown in minutes after initiation of illumination.

illuminated cultures compared to dark grown cultures (Fig. 1). Results of the quantitative analysis of blue light induction of pCon-5 are shown in Fig. 2. The results of RNA dot blot analyses indicate that actively growing mycelia contain significantly more message for pCon-5 after a 5 or 10 min photoinduction. Then, during an additional twenty minutes of dark incubation, the pCon-5 transcript level reached its maximum and subsequently dropped off sharply during a further 30 min dark incubation (Fig. 2). This decrease in the mRNA amount between timepoint 30 and 60 min reflects RNA turnover rather than non-production of pCon-5 specific transcripts since mycelia grew only slightly under conditions we used for light / dark incubation. The dry weight of a mycelial pad increased only 10–15% between the onset of light and the end of the longest incubation period (60 min) (data not shown).

Previous experiments have shown that pCon-5 plasmid DNA hybridize to three different mRNA species of approximates size of 1.15, 1.45 and 1.75 kilobases (kb) (9). The most abundant conidiation-inducible transcript is 1.15 kb (9). By definition (19) this transcript is complementary to the gene *con-5*. Northern analysis (Fig. 3) showed that a mRNA species of 1.15 kb accumulated in response to illumination. Since transcripts of the same size were conidiation- and light-inducible, we assumed that they belong to the same mRNA species; which is complementary to *con-5*.

For the con-8, con-10 and *con-13* genes, only *con-10* specific RNA accumulated in response to 10 min of blue light illumination (Fig. 1). The blue light induction profile of *con-10* was very similar to that of *con-5* (Fig. 2). Transcript levels of *con-5* and *con-10* did not increase following exposure to 30 min red light (Fig. 1 and 2).

Photoinducibility of *con-5* and *con-10* was also investigated in the nonphotoresponsive *wc* mutants (22). The products of the *wc* genes are known to be required for light induced increases of transcript amounts for all photoregulated genes isolated so far (10, 11, 24; Lauter, Schmidhauser, Yanofsky, Russo unpublished results). Two alleles of *wc-1* and *wc-2* (see Material and Methods) were tested for photoinducibility of *con-5* and *con-10* ; no RNA accumulation was observed following 60 min white light illumination (Fig. 4).

Under our growth conditions pCon-3 message accumulation was induced between 30 and 60 min after harvesting the mycelia and therefore after the transfer from submerged to aerobic conditions (Fig. 1). This transfer induces conidiation (23). Berlin and Yanofsky reported that pCon-3 mRNA accumulates 2 hours after the induction of conidiation (9). The studies presented here lead to the determination of an earlier timepoint (range) for expression. pCon-3 was the earliest 'transfer-induced' *con* gene. A quantitative analysis showed that changes in transcript levels of pCon-3 were light-independent.

DISCUSSION

Studies described here demonstrate that two *con* genes, *con-5* and *con-10*, are regulated by light and one *con* gene, pCon-3, is activated very early during conidiation. The developmental process of conidiation starts with the formation of aerial hypae which are detectable 1 to 2 hours after induction of conidiation (1, 23). pCon-3 specific transcripts accumulated between 30 and 60 min after induction of conidiation. Gene inactivation experiments should show whether the changes in pCon-3 mRNA levels were accidentally parallel to the morphological transformations associated with aerial hyphae formation or whether the product of this gene is essential for this differentiation process.

Transcription of *con-5* and *con-10* was observed to be under both developmental and blue light control. *con-5* and *con-10* shared this property with two genes that encode enzymes essential for carotenoid anabolism in *Neurospora crassa, al-1* and *al-2*. The functions of the *con* gene products are unknown. Because *con-5* and *con-10* transcript levels are increased in response to the same two environmental challenges as *al-1* and *al-2*, one can not exclude the possibility that these *con* genes are involved in carotenoid biosynthesis. On the other hand, it is equally likely

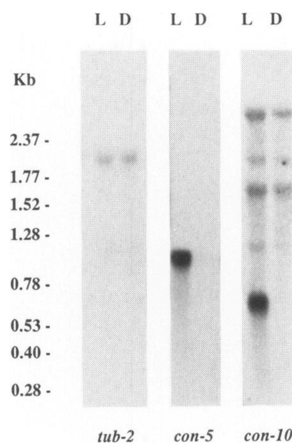

Fig. 3. Identification of transcripts complementary to *con-5* and *con-10* differentially expressed in illuminated cultures. Mycelia for RNA isolation were irradiated with white light for 30 min. RNA was extracted and 10 μg of total RNA per sample was separated by electrophoresis through a 1.5% agarose-formaldehyde gel and then transferred to Gene Screen. RNA was probed with radiolabeled pCon-5, *con-10* and *tub-2* DNA. The gene *tub-2* encodes *N. crassa* β-tubulin (28). Transcriptional initiation of *tub-2* is known to be light-independent (10, 11). The extent of hybridization was visualized by autoradio-graphy. Symbols: L, 30 min white light; D, 30 min dark.

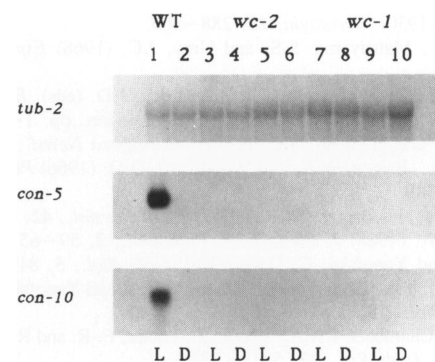

Fig. 4. Light-induced accumulation of *con-5* and *con-10* transcripts in *wc-1* and *wc-2* mutant strains. Two alleles of both *wc-1* and *wc-2* were tested. Wild type and *wc* mycelia for RNA isolation were irradiated with white light for 60 min. RNA was extracted and 10 μg of total RNA per sample was separated by electrophoresis through a 1.5% agarose-formaldehyde gel and transferred to Gene Screen. RNA was probed with radiolabeled *tub-2*, pCon-5 and *con-10* DNA. The extent of hybridization was visualized by autoradiography. Symbols: L, 60 min white light; D, 60 min dark; 1–2, wild type (STa); 3–4, *wc-2* (FGSC 4408); 5–6, *wc-2* (R 251); 7–8, *wc-1* (FGSC 4398); 9–10, *wc-1* (FGSC 4396).

that the products of *con-5* and *con-10* are involved in those steps of asexual differentiation which are light responsive (2–8). Gene inactivation experiments should increase our understanding of the functions of the *con-5* and *con-10* protein products.

In response to the environmental stimulus blue light *con-5* and *con-10* showed a mRNA induction profile that was very similar to that of *al-1* (10), *al-2* (Lauter, Schmidhauser, Yanofsky, Russo unpublished data), *bli-3* and *bli-4* (11). Although the amplitude of the blue light induction curve varied from a factor of 5, in case of *con-10*, to a factor of 90, in case of *bli-3* and *bli-4* (11), the shape of the curve was the same for all these genes. Therefore, regarding blue light regulation, it may be possible to classify *al-1, al-2, bli-3, bli-4, con-5* and *con-10* into a single responding group.

Photoinduced RNA accumulation was not observed in the regulatory mutants *wc-1* and *wc-2* . The mechanism of photoregulation might be the same for the very similar regulated genes *al-1, al-2, bli-3, bli-4, con-5, con-10,* although they are 'in toto' not physically linked. *al-1* and *al-2* are located on *Neurospora crassa* chromosome I (25), *con-5* and *con-10* on chromosome IV (9), *bli-3* on chromosome IV and *bli-4* on chromosome II (26).

With the discovery of light induction of the *con* genes, nine photoinducible genes have been identified so far in *Neurospora crassa* (10, 11, 24). Further analysis with these genes should increase our understanding of gene regulation by blue light.

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