Rapid Blue Light Regulation of a *Trichoderma harzianum* Photolyase Gene*

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Photolyases and blue light receptors belong to a superfamily of flavoproteins that make use of blue and UVA light either to catalyze DNA repair or to control development. We have isolated a DNA photolyase gene (phr1) from *Trichoderma harzianum*, a common soil fungus that is of interest as a biocontrol agent against soil-borne plant pathogens and as a model for the study of light-dependent development. The sequence of phr1 is similar to other Class I Type I eukaryotic photolyase genes. Low fluences of blue light rapidly induced phr1 expression both in vegetative mycelia, which lack photoprotective pigments, and, to a greater extent, in conidiophores. Thus, visible light induces the development of pigmented, resistant spores as well as the expression of phr1, perhaps announcing in this way the imminent exposure to the more damaging short wavelengths of sunlight. Light induction of phr1 in non-sporulating mutants shows that a complete sporation pathway is not required for photoregulation. The light requirements for photoinduction of phr1 were not altered in *dimY* photoreception mutants. This suggests that photoinduction of sporulation and of photolyase expression is distinct in their photoreceptor system or in the transduction of the blue light signal.

Since its origin, life has been a continuous fight against the harmful effects of UV light. Photoreactivation is an efficient repair mechanism for cyclobutane pyrimidine dimers (CPDs),\(^1\) the most common type of DNA damage induced by UV light. It is mediated by photolyase (EC 4.1.99.3), an enzyme that uses blue and UVA light (320–400 nm) to split dimers (1). Photolyase absorbs light due to the presence of two intrinsic chromophores: a catalytic pigment, reduced flavin, and a light-harvesting pigment that is either a deazaflavin (8-hydroxy-5-deazaflavin) or a pterin (MTHF). CPD photolyases are present in the three kingdoms of life, but two classes, distinctly related, can be distinguished: the Class I or microbial CPD photolyases and the Class II or CPD photolyases from higher eukaryotes (2). Based on the kind of light-harvesting pigment, Class I photolyases are subdivided in two types: Type I or the MTHF class and Type II or the 8-hydroxy-5-deazaflavin class. CPD photolyases belong to the emerging photolyase/blue light receptor family, which also includes (6-4) photolyases and blue light photoreceptors. (6-4) photolyases repair (6-4) photoproducts. Blue light receptors sense light in plant seedlings and trigger responses such as inhibition of hypocotyl elongation and phototropism (3, 4). All members of the family have chromophores for light absorption, but only CPD and (6-4) photolyases have repairing activity. These different proteins have probably evolved by a divergent process from a common ancestor that had a primarily CPD photolyase function (5, 6). As photoreactivation is separated from photoreception in photosynthetic organisms, it has been proposed that fungi, non-photosynthetic bacteria, and lower eukaryotes with blue/UVA morphogenetic responses constitute suitable candidates in which to search for an ancestral photolyase that would also function as a photoreceptor (5). However, a *Neurospora* mutant in which the photolyase gene was disrupted (7) exhibits a full response to the light stimulus.

In view of the DNA protective role of photolyase, it seems likely that photoreactivation might be increased by exposure to any environmental factors that are harmful to DNA. In this sense, it has been reported that UVB light (280–320 nm) induces photoreactivation in *Arabidopsis* (8). It has also been reported that UVC (200–280 nm) and chemical mutagens induce photolyase expression in *Escherichia coli* and yeast (9, 10). Furthermore, visible light induces photoreactivation in goldfish cultured cells and in *Phycomyces* spores (11, 12). Visible light also induces photolyase gene expression in goldfish cell cultures (13). Thus, visible light could be acting as a cue that is associated with the more damaging UV regions of the spectrum; it has been proposed that blue light responses evolved to alert organisms to impending stress (14).

Although photoreactivation has been studied extensively, the role of photolyase and its developmental regulation in different stages of eukaryotic life cycles is poorly understood. According to its function, more photolyase activity would be expected upon transitions from dark to light or during continuous exposure to light. In fact, high levels of photolyase transcription in *Drosophila* ovaries were found and related to survival in the embryonic stage (15). To test the hypothesis that associates photolyase with photomorphogenesis and development, we decided to study the function of a photolyase in *Trichoderma harzianum*. This common soil fungus is used as a biocontrol agent of some phytopathogenic fungi (16) and as a photomorphogenetic model due to its ability to comitadise upon...
exposure to light. In total darkness, T. harzianum grows indefinitely as a mycelium provided that nutrients are not limiting. However, a brief pulse of blue light given to a radially growing colony induces synchronous sporulation. A ring of conidiophores bearing green conidia is produced at what had been the colony perimeter at the time of the light pulse (17). A cryptochrome-type photoreceptor is involved in this response (18). The action spectrum of photoreactivation in T. harzianum corresponds to a photolyase of the MTHF class (19). Here we report the cloning of a T. harzianum photolyase gene (phr1), its strong and rapid induction by low fluences of blue light, and a subtle but significant developmental regulation during sporulation.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—T. harzianum (ATCC21737) was grown at 24–25 °C on complete PDYC medium (24 g/liter potato de- trose broth, 2 g/liter yeast extract, and 1.2 g/liter casein hydrolysate; all from Difco). Some experiments employed the dimY mutants LS44 (lys-133W5 dimY) and R10 (rib-Br dimY) and their corresponding parental strains, LS (lys-133W5) and RB (rib-Br) (20). Two non-sporulating mutants (NS1 and NS2) and their parental strains were also used. Mutants NS1 and NS2 were isolated from strain 43arg-Y1 kindly provided by Dr. D. Avivi) following mutagenesis with x-rays for 40 min at 270 R/min, giving a survival rate of 23%.

Isolation of phr1—The completely degenerate primers PL1 (18-mer, 5′-GACTTGG(C/A)NGACC(T/C)TT(T/C)TA(T/C)-3′) and PL3 (20-mer, 5′-GG(A/G)TT(G/A)ANACNC(G/T)(G/A)AA(G/A)TA-3′) were synthesized (Biotechnology General, Rehovot, Israel) according to the amino acid sequences indicated in Fig. 1. These primers were used to PCR-amplify a fragment using a program including 2 min at 95 °C, followed by 45 cycles for 1 min at 94 °C, 45 s at 45 °C, and 2 min at 72 °C. Thermocycling was followed by an additional extension step of 10 min at 72 °C. A Southern blot (21) of the products was probed at low stringency with the Neurospora crassa phr1 cDNA clone (22). An abundant band of 438 bp, which hybridized to N. crassa phr1, was cloned in pCRIIex (Stratagene), sequenced, and used to screen genomic and cDNA libraries. Six different cDNA libraries of strains IMI206040 and ATCC32173 previously described (23, 24) were screened with the above-mentioned probe, and three clones showing similar restriction patterns were obtained. A 5.8-kb phr1 fragment was subcloned into the pT7T7 vector (Roche Molecular Biochemicals). Four fragments, 1.35 kb (EcoRV), 0.5 kb (EcoRV-HindIII), 1.2 kb (HindIII-EcoRV), and 1 kb (BglII), from the 5.8-kb original clone were subcloned into the Pzero vector (Stratagene). Sequencing was carried out in an Applied Biosystems Prism 377 DNA sequencer using a dye terminator cycle sequencing kit with AmpliTag polymerase. The amino acid alignment was obtained with the MEGALIGN program (DNASTAR, Inc., Madison, WI) according to the CLUSTAL method (26).

Photoinduction—Colonies were grown on filter paper soaked with PDYC medium: an 8-cm Whatman No. 50 disc overlaying a 7-cm Whatman No. 1 disc in a 9-cm plastic Petri dish. Experiments were begun after 36 h of growth in total darkness, when colony diameter reached 40–50 mm. For the non-sporulating mutants NS1 and NS2, the upper filter paper was replaced with a layer of dialysis tubing, and the colonies were grown for 4 days in the dark. The cultures were photoinduced by exposure to a standard blue source consisting of light from a cool-white fluorescent tube filtered through a blue acrylic photoinduced by exposure to a standard blue source consisting of light from a cool-white fluorescent tube filtered through a blue acrylic

RESULTS

Isolation of a T. harzianum Photolyase Gene—Using degenerate primers targeted to conserved regions of photolyases, a 438-bp fragment was PCR-amplified from Trichoderma DNA. Sequence analysis of this fragment exhibited 82.4% identity, at the amino acid level, to N. crassa phr1, thus confirming that it indeed encodes a member of the photolyase family.

Screening a cDNA library with the 438-bp probe resulted in the isolation of partial clones (600 bp) containing the 3′-end. A 5.8-kb Phr1 fragment, from recombinant phage isolated by screening a genomic library with the above-mentioned probe, was subcloned. Comparison between the restriction patterns from the 5.8-kb fragment and the cDNA clones allowed us to localize the coding sequence in a 3′-kb region. The 3′-kb fragment sequence contains an open reading frame of 1887 bp, encoding a polypeptide of 629 amino acids, interrupted by a single intron of 59 bp at bp 2389. The gene was named phr1. The 1887-bp open reading frame has three potential initiation codons. Only the first ATG codon has a nucleotide sequence (TGTAATAGCT) in its vicinity that matches the consensus sequence (C/T)CAA(A/C)ATA(A/T)C/CT) reported around initiation codons of T. harzianum (23–25, 28).

The overall identity of the T. harzianum phr1 deduced amino acid sequence to the N. crassa and Saccharomyces cerevisiae photolyases is 62 and 30.6%, respectively. The sequence identity in the helical domain, where most amino acids involved in chromophore binding and substrate interaction are located, is 73.3 and 45.1%, respectively (Fig. 1). The deduced Phr1 sequence has all the amino acids involved in FAD binding, present in all photolyases and blue light receptors. Like Class I CPD photolyases, it conserves the amino acids that interact with CPD, and it shares with Class I CPD photolyases the amino acids involved in MTHF binding (Fig. 1) (6, 29). On the basis of identity to fungal photolyases and the presence of the conserved amino acids involved in MTHF binding, phr1 can be assigned to the Class I Type I photolyase group (2).

The transcription start point was determined by primer extension, and it is located 45 bp upstream of the first ATG codon (Fig. 2, A and B). Two other potential transcription start points were also detected 29 and 36 bp downstream of the first ATG codon (Fig. 2A). Analysis of the 5′-upstream region shows the presence of several putative regulatory sequences. These are a TATA-like box sequence at position −16; CCAAT boxes at positions −127 and −241; a DNA damage-responsive element (called a DRE box) at position −365; an APE element, involved in blue light regulation of the N. crassa al-3 gene (31), at position −86; and binding sites for Stunted (StuA), an Aspergillus nidulans protein involved in conidiophore development (32), at positions −41 and −822. Other putative control elements found include binding sites for CreA (30) at positions −136 and −215; two GATA boxes (34) at positions −73 and +19; and heat shock factor boxes at positions −28, −388, −823, and −839. Some of these boxes are indicated in Fig. 2B. The sequence CCTTGCGCTTT that resembles the con-10 dark repression-related sequence (35) is present twice in the phr1 promoter at positions −25 and −536. The 3′-untranslated region contains a polyadenylation site at position +2071, obtained by alignment with the cDNA sequences. The calculated size of the transcript is 2 kb, in agreement with the 2.2 kb estimated by Northern blotting. Phr1, like other eukaryotic...
CPD photolyases, has a protruding N-terminal domain with putative nuclear localization signals (KGSK and KRVK) and the motif RRFYPH, which is a putative signal for mitochondrial localization (Fig. 1) (22).

Steady-state Levels of phr1 mRNA Increase following Exposure to Light and during Development—For analysis of the expression of phr1 during photoinduced conidiation, vegetative mycelium was grown in the dark until it reached a conidiation-competent stage and then was exposed to a pulse of blue light of 540 μmol m⁻² s⁻¹. Under these conditions, conidiation is triggered at the edge of the colony, and a ring of conidiophores and mature spores is visible 24 h later (17). No phr1 mRNA was detected in the dark, becoming detectable immediately after the light pulse (Fig. 3B). The message reached its maximum accumulation between 15 and 30 min and started to decrease 60 min after the pulse. From 2 to 8 h after the light pulse, phr1 mRNA continued decaying. Similar induction kinetics were observed when the cultures were exposed to continuous illumination.³ An additional, weaker signal was detected, of smaller size than the phr1 mRNA. This band may be a degradation product that is trapped by ribosomal RNA, although we cannot exclude the possibility that it could correspond to another transcript. The rapid and transient accumulation of the phr1 transcript after the light pulse (Fig. 3B) strongly suggests that it is directly triggered by light. The time window when phr1 expression is strongly induced can be correlated with other physiological events that occur in photoinduced sporulation. The peak of induced phr1 expression occurs prior to the earliest sign of visible morphogenesis, represented by stabilization of aerial hyphae, which occurs between 2 and 4 h after the light pulse (Fig. 3A). Thus, phr1 expression is an early marker for photo-

³ G. Berrocal-Tito, unpublished results.
induction. Previous experiments indicated that a blue light exposure of $125 \pm 16 \mu mol m^{-2}$ was sufficient for half-saturation of \( \text{phr}1 \) induction, whereas 90% saturation was obtained with 240 \( \mu mol m^{-2} \), as determined from nonlinear fits to fluorescence curves. Experiments carried out with wide-band filters to determine the range of wavelengths active in \( \text{phr}1 \) induction showed that blue light was more efficient. \( \text{phr}1 \) induction levels by 420 \( \mu mol m^{-2} \) of green and red light were 65 and 0% relative to blue light induction, respectively.

In blue light-induced conidiating cultures, conidiophore rings were harvested starting 12 h after the light pulse, when phialides are emerging from branched conidiophores (Fig. 3A). At this stage, a weak signal of \( \text{phr}1 \) mRNA was detected from whole colonies, which persisted through 16–24 h after the light pulse, during completion of conidiophore development and the formation of pigmented spores. To test whether \( \text{phr}1 \) expression is developmentally regulated, we analyzed separately conidiophore rings (c) and the surrounding mycelia (m) and found that conidiophore rings have more \( \text{phr}1 \) mRNA than mycelia at the times sampled from 12 to 24 h after the light pulse (Fig. 3B). This suggests that \( \text{phr}1 \) is also up-regulated during conidiophore development. A weak band above the \( \text{phr}1 \) mRNA signal appeared in samples from 12 to 24 h (Fig. 3B) and also appeared in dark controls (Figs. 4 and 6B); this corresponded to a transcript with some homology to \( \text{phr}1 \) that seems to be repressed by light.

Light-induced Expression of \( \text{phr}1 \) Is Due to Transcriptional Activation and Does Not Require Protein Synthesis—The high levels of \( \text{phr}1 \) mRNA detected upon illumination may be the result of transcriptional activation or increased stability of the \( \text{phr}1 \) message. For this reason, we tested the effect of actinomycin D on the light-induced accumulation of \( \text{phr}1 \) mRNA. Inhibition of transcription initiation by actinomycin D (AD) prevented accumulation of the message (Fig. 4), indicating that the increase in the \( \text{phr}1 \) mRNA level is mainly due to transcriptional activation. In addition, inhibition of protein synthesis by cycloheximide (CH) did not block \( \text{phr}1 \) photoinduction, indicating that protein synthesis is not needed for transcriptional activation of \( \text{phr}1 \) (Fig. 4).

\( \text{phr}1 \) Is Regulated by Light in Conidiophores and Vegetative Mycelia—To test whether \( \text{phr}1 \) can be light-induced in conidiophores, conidiating cultures at 12, 16, 24, and 36 h after photoinduction were exposed to continuous illumination (3 \( \mu mol m^{-2} \) s$^{-1}$) 15 min before harvesting of conidiophores. Under these conditions, the \( \text{phr}1 \) transcript accumulated strongly in conidiophore-enriched samples as compared with the dark controls (Fig. 5B). To compare \( \text{phr}1 \) photoinduction in conidiophores (c) with that in the mycelium, the mycelial region surrounding the conidiophore ring (m), including the central (cm) and peripheral (pm) mycelia, was collected. Both the conidiophore and mycelium showed an increase in \( \text{phr}1 \) mRNA levels in light-exposed conidiating cultures compared with their dark controls, but conidiophore samples showed a stronger signal than the mycelium (Fig. 5B). As these results suggested a different capacity for photolyase induction, we analyzed this effect in more detail. Induction of \( \text{phr}1 \) was compared between conidiophore rings, newly formed conidiation-competent pe-
Blue Light Induction of a Fungal DNA Photolyase Gene

The predicted sequence of phr1 contains the features of a functional photolyase (Fig. 1). Furthermore, it does not have the C-terminal extension that is found in several blue light photoreceptors, but not in functional CPD photolyases. The prediction of the first ATG codon as the start codon in the deduced Phr1 protein was consistent with primer extension analysis. It differs from the N. crassa gene, in which the second ATG codon was reported as the translation start site. Thus, the Trichoderma photolyase is 38 amino acids longer than the N. crassa photolyase at its N terminus. To gain some insight as to the possible function of this extra region in Phr1, it was compared with the SBASE library of protein domain sequences, indicating best similarity to the signal peptide of the clostridial leader, and goldfish blue light-sensitive opsins domains (36). These results suggest a translocating or interacting function for this region of Phr1.

The presence of nuclear and mitochondrial localization signals in Phr1 (Fig. 1) suggests that it repairs dimers in both compartments, as demonstrated in yeast (37). The Class I Type I CPD photolyase predicted from the phr1 sequence can account for the photoreactivation of UV-inactivated spores (19). All efforts to detect additional photolyase genes, by using low stringency Southern analysis, PCR, and complementation experiments of E. coli phr1- mutants with several Trichoderma cDNA libraries, did not lead to cloning of additional genes. This suggests that there are no additional photolyase genes in T. harzianum. Thus, we propose that the phr1 gene product is responsible for the photoreactivation of UV-inactivated Trichoderma conidia.

Visible light induction of photoreactivation seems to be a phenomenon common to all vertebrates (14), suggesting that the induction of repair mechanisms by long wavelengths of light may be an adaptive response to the environment, in which visible light components are acting as a cue for harmful effects of the UV component of sunlight. Increased photolyase expression induced by visible light in goldfish cell cultures was observed 4–8 h after exposure to light equivalent to 100 kJ m⁻² (38). Up-regulation in this system was also induced by peroxide and by conditions of growth arrest (39). It was therefore proposed that a subtle DNA damage caused by visible light may be the signal for photolyase induction and that oxygen radicals may be involved in triggering photolyase expression. In contrast, the Trichoderma photolyase gene is induced by fluences of blue light as low as 20 μmol m⁻² (Fig. 5C). Furthermore, phr1 mRNA is detectable even at “zero time,” immediately after the light pulse (Fig. 3). Thus, the blue light-induced expression of the Trichoderma photolyase is most likely directly regulated by light rather than by a DNA damage mechanism. Indirect evidence for the latter is that, in addition to the existence of a blue light-regulated element, there is a DNA damage-responsive element in the phr1 promoter, suggesting that both mechanisms work independently.

The levels of phr1 mRNA in dark-grown mycelia are undetectable, consistent with the fact that Trichoderma mycelium, which is hyaline, develops in soil, where UVC levels are virtu-

Characterization of phr1 Induction by Light in Non-sporulating and Photoreception Mutants—As phr1 is up-regulated both by light and during development, we tested whether photoinduction would occur under conditions in which morphogenesis is blocked. In two non-sporulating mutants (NS1 and NS2), phr1 expression was induced by a pulse of blue light (Fig. 6A). The non-sporulating mutants had a general decrease in mRNA content as compared with their parental strains, as shown by hybridization with an actin gene (not regulated in the time window of the experiment) (Fig. 6A). In addition, we tested whether photoinduction would occur under conditions in which photoreception is altered. dimY photoreceptor mutants (LS44 and RB10) were compared with the strains from which they were derived. In this case, phr1 expression was induced by exposure to blue light that saturated the induction of conidia-
ally zero. It has been proposed that blue light acts as a stress signal, alerting the organism to impending photodamage (14). According to this light-adaptive response theory, the transient and strong \textit{phr1} mRNA increase caused by illumination can be explained as a mechanism designed to turn on a system for protection against any potential light-induced damage. The remaining levels of \textit{phr1} mRNA detectable even after completion of conidiation could be correlated with the enzyme levels able to repair potential DNA damage.

Molecular correlations with photoinduced conidiation of \textit{Trichoderma} include a decrease in expression of \textit{pgk} (phosphoglycerate kinase) (28), \textit{gpdh} (glyceraldehyde-phosphate dehydrogenase) (24), and \textit{actin} (28) and an increase in a highly expressed gene (\textit{con-1}) and in a multidomain cell-surface protein and its mRNA (2). All these changes occur in the later stages of blue light-induced conidiation (12 h after the light pulse). As \textit{phr1} induction occurs within the first minutes after the light pulse, \textit{phr1} can be considered the first light-regulated gene isolated in this organism.

In \textit{N. crassa}, the genes \textit{al-1}, \textit{al-2}, and \textit{al-3}, which code for carotenogenesis enzymes, are induced shortly after a brief illumination, and their transcript levels reach a maximum in ~30 min. Similar patterns of induction are shared by the conidiation genes \textit{con-5}, \textit{con-6}, and \textit{con-10} and the blue light-induced genes \textit{bli-3} and \textit{bli-4}. All these genes are considered as a fast responding group (7). Deletion analysis of the \textit{al-3} promoter indicated that the APE and CCAAT boxes are responsible in part for the response to light. The putative APE element was also found in \textit{al-1}, \textit{con-10}, and \textit{ccg-2} (31). Expression of fast light-regulated genes in \textit{Neurospora} requires functional WC-1 and WC-2, putative transcription factors. Recent evidence that the WC-1 zinc finger binds to GATA boxes emphasizes the importance of these sequences in light inducibility (40).

A sequence similar to the APE element is present in the \textit{phr1} promoter (Fig. 2B). The \textit{phr1} APE sequence GAA-TTGGCG is 6 out of 8 bases identical to that of the \textit{al-3} gene. The APE element is flanked by CCAAT, GATA, and TATA-like boxes. Comparison of \textit{al-3} and \textit{phr1} promoter sequences shows a conservation between elements and their arrangement (Fig. 7). Considering the similarities between the \textit{phr1} and \textit{al-3} promoters, we can also expect similarities in their regulatory mechanisms. Indeed, \textit{Trichoderma} could be an additional model in which to test if a regulatory protein binds the APE element.

\textit{phr1} mRNA levels are higher in conidiophores developed in the dark than in the surrounding mycelium (Figs. 3 and 5B, lower panel). This indicates that \textit{phr1} mRNA is synthesized de novo or that it is more stable in conidiophores. The presence of the Stunted A protein binding box in the \textit{phr1} promoter adds support to the first mechanism, suggesting a possible regulation of photolyase during conidiophore development. If this is true, this regulation should also be observed in conidiophores from cultures induced to conidiate by stimuli other than light.

Developing and mature conidiophores were able to increase the \textit{phr1} mRNA in response to light in a way similar to the mycelium. The level of \textit{phr1} mRNA, however, was more induc-
induced conidiation, suggesting that CPDs, but does not repair them (41). Moreover, it has been suggested that the response depends on particular developmental states of the cell. The photoinduction time courses of conidiophores and peripheral mycelium are similar3; therefore, their differential responsiveness is not due to different kinetics. The fact that conidiophores and new conidiation-competent mycelium are more responsive to phr1 induction than central mycelium suggests a relationship between photomorphogenesis and phr1 induction.

phr1 photoinduction in conidiation-competent mycelium is tightly correlated with photoinductive events in blue light-induced conidiation, suggesting that phr1 photoinduction could be part of this event. Photolyase binds adducts other than CPDs, but does not repair them (41). Moreover, it has been shown in non-sporulating mutants. Therefore, we could extrapolate that photolyase could bind visible light-damaged DNA. Transient phr1 photoinduction drives a photolyase increase, which could result in a distorted DNA-photolyase complex accumulation, perhaps acting to amplify the light signal in the cell. Although phr1 appears to be developmentally regulated, its light inducibility seems to be independent of the capacity of the fungus to complete the conidiation process, as shown in non-sporulating mutants.

Photolyase expression might be induced by the same photoreceptor(s) responsible for the photoinduction of conidiation. In this case, one would expect induction of phr1 to be decreased in the same manner as conidiation in mutants belonging to the dimY complementation group. These mutants require increased fluences to induce conidiation. They also have altered action spectra for the induction of conidiation and altered in vivo absorption spectra (20). On the other hand, photoreactivation was normal in dimY mutants, indicating that they have a functional photolyase (19). Comparison of induction by pulses that are saturating (480 μmol m⁻²) or subsaturating (20 μmol m⁻²) for conidiation shows that the dimY mutations affect sporulation, but not phr1 induction. The data for phr1 mRNA induction do not indicate an increased requirement for light in the mutants. Furthermore, fluence response analysis of phr1 induction indicates that twice more light is required for half-saturation of phr1 induction than for conidiation. Normal sensitivity to light for phr1 induction in dimY strains is most easily explained by different photoreceptors for sporulation and photolyase expression. This conclusion, however, is still limited by our lack of knowledge of the molecular nature of dimY. It remains possible that the same photoreceptor is involved in both responses, but that the transduction steps for phr1 induction are distinct from those leading to conidiation and do not depend directly on dimY.

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REFERENCES

1. Sancar, G. (1988) Annu. Rev. Biochem. 57, 29–67
2. Yasui, A., Eker, A. P. M., Yasuhira, S., Yamaizuru, H., Kohayashi, T., Takao, M., and Okawa, A. (1994) EMBO J. 13, 6143–6151
3. Ahmad, M., and Cashmore, A. R. (1993) Nature 366, 162–166
4. Lin, C., Ahmad, M., Chan, J., and Cashmore, A. R. (1996) Plant Physiol. (Bethesda) 110, 1047
5. Horwitz, B., and Berrocal, G. (1997) Bot. Acta 110, 360–368
6. Kanai, S., Kikuno, R., Toh, H., Ryo, H., and Todo, T. (1997) J. Mol. Evol. 45, 781–787
7. Linden, H., Ballario, P., and Macino, G. (1998) Fungal Genet. Biol. 22, 141–150
8. Pang, Q., and Hays, J. B. (1991) Plant Physiol. (Bethesda) 95, 536–543
9. Hori, M., Yamamoto, K., and Ohmishi, T. (1987) Mol. Gen. Genet. 209, 200–202
10. Sebastian, J., Kraus, B., and Sancar, G. B. (1990) Mol. Cell. Biol. 10, 4630–4637
11. Yasuhira, S., Mitani, H., and Shima, A. (1991) Photochem. Photobiol. 53, 211–215
12. Gaillard, P. (1996) Microbiol. Res. 151, 1–9
13. Mitani, H., and Shima, A. (1995) Photochem. Photobiol. 61, 373–377
14. Gressel, J., and Rau, W. (1983) in Photomorphogenesis: Encyclopedia of Plant Physiology (Shropshire, W., and Mohr, H., eds) Vol. 16B, pp. 603–639 Springer-Verlag, Berlin
15. Todo, T., Ryo, H., Takemori, H. Toh, H., Nomura, T., and Kondo, S. (1994) Mutat. Res. 315, 213–228
16. Papavizas, G. C. (1985) Annu. Rev. Phytopathol. 23, 23–54
17. Gressel, J., and Galun, E. (1987) Dev. Biol. 157, 555–598
18. Gressel, J., and Hartman, K. M. (1968) Planta (Heidelberg) 79, 271–274
19. Zamez-Baron, L., Berrocal-Tito, G. M., Amit, R., Herrera-Estrella, A., and Horwitz, B. (1997) Photochem. Photobiol. 65, 849–854
20. Horwitz, B. A., Gressel, J., and Malkin, S. (1985) Curr. Genet. 9, 605–613
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Yajima, H., Inoue, H., Okawa, A., and Yasui, A. (1991) Nucleic Acids Res. 19, 5359–5362
23. Carcelin, C., Gutierrez, A., Jimenez, B., Van Montagu, M., and Herrera-Estrella, A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10903–10907
24. Puyeski, M., Ponce-Noyola, P., Horwitz, B., and Herrera-Estrella, A. (1997) Microbiology 143, 3157–3164
25. Geremia, R. A., Goldman, G. H., Jacobs, D., Arildes, W., Vila, S. B., Van Montagu, M., and Herrera-Estrella, A. (1993) Mol. Microbiol. 8, 603–613
26. Higgins, D. G., and Sharp, P. M. (1988) Gene (Amst.) 73, 257–244
27. Jones, J. D. G., Dunsmuir, P., and Bedbrook, J. (1985) EMBO J. 4, 2411–2418
28. Goldman, G. H., Geremia, R. A., Caplan, A. B., Vila, S. B., Villaroel, R., Van Montagu, M., and Herrera-Estrella, A. (1992) Mol. Microbiol. 6, 1231–1242
29. Park, H.-W., Kim, S.-T., Sancar, A., and Deisenhofer, J. (1995) Science 268, 1866–1872
30. Jang, Y. K., Ji, Y. H., Shim, Y. S., Kim, M. J., Yoo, E. J., Choi, I. S., Lee, J. S., Seong, R. H., Hong, S. H., and Park, S. D. (1996) Mol. Gen. Genet. 253, 167–175
31. Carato, A., Cogoni, C., Morelli, G., and Macino, G. (1994) Mol. Microbiol. 13, 787–795
32. Dutton, J. R., Johns, S., and Miller, B. L. (1997) EMBO J. 16, 5710–5712
33. Mathieu, M., and Felenbok, B. (1994) EMBO J. 13, 4022–4027
34. Marzluf, G. A. (1997) Microbiol. Mol. Biol. Rev. 61, 17–32
35. Corrochano, L. M., Lauter, P. R., Ebhose, D. J., and Yanoiski, C. (1995) Dev. Biol. 167, 190–200
36. Henikof, S., and Henikof, J. G. (1994) Genomics 19, 97–107
37. Yasui, A., Yajima, H., Kohayashi, T., Eker, A. P. M., and Okawa, A. (1992) Mutat. Res. 276, 231–236
38. Yasuhira, S., and Yasui, A. (1992) J. Biol. Chem. 267, 25644–25647
39. Mitani, H., Uchida, N., and Shima, A. (1996) Photochem. Photobiol. 64, 943–948
40. Ballario, P., Vittori, O., Magnelli, A., Talara, C., Cabibbo, A., and Macino, G. (1996) EMBO J. 15, 1650–1652
41. Fox, M. E., Feldman, J., and Chus, G. (1994) Mol. Cell. Biol. 14, 8071–8077
42. Mitani, H. (1983) Mutat. Res. 107, 279–288
43. Sancar, G. B. (1985) Nucleic Acids Res. 13, 8231–8239
44. Betina, V. (1984) Int. J. Microbiol. 2, 55–68