Reactive Oxygen Species Affect Photomorphogenesis in Neurospora crassa*

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In Neurospora crassa, several biological phenomena such as the synthesis of carotenoids in the mycelia and polarity of perithecia are regulated by light. We found that a sod-1 mutant, with a defective Cu,Zn-type superoxide dismutase (SOD), showed accelerated light-dependent induction of carotenoid accumulation in the mycelia compared with the wild type. The initial rate of light-induced carotenoid accumulation in the sod-1 mutant was faster than that in the vvd mutant known to accumulate high concentrations. This acceleration was suppressed by treatment with antioxidant reagents. Light-induced transcription of genes involved in carotenoid synthesis, al-1, -2, and -3, was sustained in the sod-1 mutant, whereas it was transient in the wild type. Moreover sod-1 was defective in terms of light-induced polarity of perithecia. By genetic analysis, the enhancement in light-inducible carotenoid synthesis in sod-1 was dependent on the wild type alleles of wc-1 and wc-2. However, the sod-1vvd double mutant showed additive effects on the carotenoid accumulation in the mycelia. These results suggested that intracellular reactive oxygen species regulated by SOD-1 could affect the light-signaling transduction pathway via WC proteins.

Light is one of the most important environmental factors being utilized for various biological phenomena by living organisms (1, 2). Typical examples are the visual systems in animals, photosynthesis in plants, and photomorphogenesis in fungi. In Neurospora crassa, several biological phenomena are under the control of blue light. Light-induced processes are summarized as follows: (i) synthesis of carotenoids in the mycelia (3), (ii) induction of conidiation (3), (iii) formation of protoperithecia on nitrogen-deficient media (4), (vi) positive phototropism of perithecial beaks (5), (v) positioning of the beak formed on the perithecum (perithecial polarity) (6, 7), and (vi) phase shift in the circadian rhythm of conidiation (8, 9).

Much information on photoreceptors and light-inducible genes has been accumulated by genetic and molecular biological analyses to understand the molecular mechanism of these photobiological phenomena. In the search for the blue light photoreceptor in N. crassa, light-insensitive mutants, white collar (wc)1-3 and wc-2, deficient in most of these blue light responses, have been isolated (4, 10, 11). The products for these genes are photoreceptors that can activate transcription. Both possess a PAS (PER, ARNT, and SIM) domain, a zinc finger DNA binding motif, and a glutamine-rich putative transcription activation domain (12, 13). WC proteins form heterodimers presumably through their PAS domains (14, 15), having promoter binding activity of light-regulated genes in Neurospora crassa.

In Arabidopsis thaliana, three major groups of photoreceptors, phytochromes (16), cryptochromes (17), and phototropins (18), have been identified. Phototropins have two LOV (light, oxygen, and voltage) domains, and bind FMN (flavin mononucleotide) as a chromophore (19). WC-1 also has one LOV domain, which is a putative chromophore-binding site. WC-1/WC-2 complex is reported to bind FAD (flavin-adenine dinucleotide) (20).

Blue light-responsive albino genes, al-1, al-2, and al-3, which encode enzymes for the synthesis of carotenoids (21–23), conidiation (con) 10 related to photo-conidiation (24) and blue light inducible genes bli-4 and bli-6 are transcriptionally activated after blue illumination of mycelia (25). No light-induced accumulation in al-1, al-2, al-3, or con-10 transcripts in the mycelia was observed in either wc-1 or wc-2 mutants (21, 23, 24, 26). WC proteins have the capacity to bind the promoter element, GATA motif, of the al-3 gene in vitro (12, 13).

In light-inducible carotenogenesis in N. crassa, there is a phenomenon called adaptation (27). In the wild type, al-3 mRNA is transiently expressed in the mycelia under light. The accumulation of al-3 mRNA reached a peak at 30 min after illumination, and decreased to the level in darkness after 1 h even in the presence of light. For this light adaptation, VIVID (VVD) protein, a candidate for a photoreceptor possessing LOV domain has been suggested to function as a negative regulator of WC-1 protein (28, 29). WC proteins play a critical role in the blue light signal transduction and light responses, such as carotenoid synthesis in the mycelia through transcriptional activation (30).

However, Dragovic et al. (31) showed that the wc-1, wc-2, and wc-1 wc-2 mutants showed self-sustained rhythmic banding of conidiation and resetting by light. These results indicated that there is a novel light receptor other than WC-1 and WC-2 in N. crassa.

In Fusarium aquaeductuum, the accumulation of carotenoid increased in the mycelia treated with methylene blue or toluidine blue under red light, although the fungus was thought to be insensitive to this spectrum of light (32). In Phaffia rhodozyma, singlet oxygen induced the carotenoid synthesis (33). These results suggested a relation between the intracel-

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‡ The abbreviations used are: wc, white collar; NDK-1, nucleoside diphosphate kinase-1; ROS, reactive oxygen species; SOD, superoxide dismutase; RT, reverse transcriptase; Pipes, 1,4-piperazinediethanesulfonic acid.
lular redox state and light-induced carotenogenesis. Previously, we have isolated nucleoside diphosphate kinase-1 (NDK-1), as a novel candidate for a light signal-transducing factor other than WC proteins in N. crassa (7, 34). The ndk-1PH278 mutant with reduced protein kinase activity showed a deficiency in the light response for perithelial polarity.

In the present study, we investigate reactive oxygen species (ROS) generation by light in Neurospora as factors to control redox state functioning as controlling factors for redundant pathways of light signal transduction. To test these possibilities, we tried to address the relationship between photomorphogenesis and ROS using a sod-1 mutant of N. crassa, defective in Cu/Zn-superoxide dismutase (Cu/Zn-SOD) (35), and found that light-induced phenomena, including carotenoid synthesis, in the mycelia of the sod-1 mutant are markedly affected. Moreover light-induced transcription of genes involved in carotenoid synthesis was sustained in the sod-1 mutant, under conditions where the expression of these genes was transient in the wild type.

**EXPERIMENTAL PROCEDURES**

*Strains of Neurospora crassa—* Strains of N. crassa used in this study were obtained from the Fungal Genetics Stock Center (Department of Microbiology, University of Kansas Medical Center, KS) and included the standard wild type; 74-OR23-1A (FGSC number 987), a Cu/Zn-superoxide dismutase (SOD) (sod-1) null mutant (sod-1) (FGSC numbers 7437 and 7435), vvd (FGSC number 7854), ur-1 (FGSC number 4445), and ur-2 (FGSC number 3818), and a sod-1 mutant was obtained after crossing of FGSC number 7437 three times into wild type; 74-OR35-1A (FGSC number 988). NDK-1 missense mutant, ndk-1PH278 (FGSC number 8416), was described by Ogura et al. (7). The ur-2 mutant (FGSC number 3818) produces a truncated WC-2 protein lacking the zinc finger motif (13, 31). Although the mutation in the ur-1 mutant (FGSC number 4445) used in this study has unknown characteristics, it is a loss of function mutant in the light-dependent carotenoid synthesis. The sod-1ndk-1PH278 double mutant was generated by crossing sod-1 (FGSC number 7437) with ndk-1PH278 (FGSC number 8416). The sod-1, ur-1 mutant was obtained by crossing sod-1 (FGSC number 7437) with ur-1 (FGSC number 4445). The sod-1 ur-2 double mutant was obtained by crossing sod-1 (FGSC number 7437) with ur-2 (FGSC number 3818). The sod-1 vvd dual mutant was obtained by crossing sod-1 (FGSC number 7437) with vvd (FGSC number 7854). In the screening of double mutants, the sod-1 mutation was detected by SOD activity assay and Northern analysis for the expression of sod-1 mRNA. Each strain was grown on a glycerol complete slant medium (36) for 1 week at 25°C.

**Analysis of Perithelial Polarity—** Perithelial polarity was analyzed as described by Oda and Hasunuma (6). The sod-1 mutant (FGSC number 7437) was incubated in darkness at 25°C on synthetic crossing medium for 5 days and fertilized with sod-1 (FGSC number 7438) conidia. After 3 days in darkness, the fertilized cultures were placed in a box with a slit 4 cm wide and illuminated directionally under 12-h light (20 μE m−2 s−1)/12-h dark regime for 2 weeks. Perithecia were observed and photographed through a dissecting microscope. In this article, perithelial polarity refers only those perithelia with the bead formed upward. Light-induced perithelial polarity is defined as the difference of those between under illumination and in darkness.

**Quantification of Mycelial Carotenoid Accumulation—** Conidia (1 × 105) were inoculated into 25 ml of Vogel’s minimal medium in 8.5-cm Petri dishes. The dishes were incubated at 30°C for 36 h in darkness, and then exposed to light (20 μE m−2 s−1) for 0.25, 0.5, 1, 2, and 3 h. Mycelia were harvested as described. In N-acetyl-l-cysteine treatment, after incubating for 36 h at 30°C, the mycelia were incubated with 1 mM N-acetyl-l-cysteine for 1 h. Mycelia were exposed to light for an additional 1 h. In the cycloheximide treatment, after the mycelia were incubated with 100 μg/ml cycloheximide, mycelia were exposed to light for 15 min. The treated mycelia were homogenized with a pestle and mortar in liquid nitrogen. Total RNA was prepared by a small-scale RNA extraction method (40). Total RNA (5 μg) was separated by glyoxal-gel electrophoresis and transferred to a piece of GeneScreen Plus membrane (PerkinElmer Life Sciences). Probes for al-1, al-2, al-3, bli-4, con-10, and 1b-tub were amplified by PCR, using N. crassa genomic DNA as a template. The primers used for PCR are as follows: al-1forward: 5’-gcatgttctgctttggcagt-3’, al-1reverse: 5’-aatcctttatccttctctct-3’, al-2forward: 5’-ccacaggaacctgtggtcggg-3’, al-2reverse: 5’-aaagctggttgcagcagctg-3’, al-3forward: 5’-agctgggtgcgtctctgcaaa-3’, al-3reverse: 5’-agctgggtgcgtctctgcaaa-3’, bli-4forward: 5’-aactagtgcctgagaagcac-3’, bli-4reverse: 5’-aatcttctcatccatctccttcctcat-3’. Hybridization with 32P-labeled probes was carried out overnight at 60°C using Church’s phosphate buffer (41). Radioisotopic signals were visualized by autoradiography using X-Omat AR film (Kodak).

RT-PCR—The absence of urc-1 mRNA was detected by RT-PCR analysis of total RNA prepared from mycelia exposed to light (20 μE m−2 s−1) for 30 min was treated with DNase I, RT-PCR grade (Invitrogen). Total RNA (5 μg) and 1 μl of oligo(dT)12–18 primer (100 pmol) were denatured in 12 μl of reaction solution for 5 min at 65°C and were annealed on ice. First strand cDNA was synthesized with the use of 1 μl of SuperScript II reverse transcriptase (Invitrogen) for 50 min at 42°C. The single-stranded cDNA was then denatured in 12 μl of 1 M riboflavin, 0.1 M sodium phosphate buffer, pH 7.8, for 15 min under room light (42). SOD activity was determined in the mycelia of the sod-1 mutant with reduced protein kinase activity showed a deficiency of those between under illumination and in darkness.

RESULTS

**Light-dependent Carotenoid Accumulation in the sod-1 Mutant—** To investigate the interaction between the response of light and ROS in N. crassa, we examined light-dependent carotenoid accumulation in mycelia of the sod-1 mutant. In the experiment to quantify mycelial carotenoid, mycelia were harvested in a growing phase prior to conidiation, because carotenoid accumulation in conidia occurs independent of the light response. The light-insensitive mutant, urc-1, was used as a negative control of light response. The sod-1 mutant showed an accelerated light-dependent induction of carotenoid accumulation in the mycelia compared with the wild type (Fig. 1). Levels of mycelial carotenoids in the sod-1 mutant were detectable after 1.5 h of illumination (Fig. 1A and B).
We quantified the \textit{uc-1} accumulation by a RT-PCR method followed by Southern blot analysis in the \textit{sod-1} and \textit{vvd} mutants. Significantly more of the light-inducible \textit{uc-1} mRNA accumulated in the \textit{sod-1} and \textit{vvd} mutant than in wild type (Fig. 2C).

**Effects of Antioxidants on Light-inducible Carotenoid Synthesis and Gene Expression**—In the \textit{sod-1} mutant, the concentration of intracellular superoxide anion radical, \(\text{O}_2^-\) is maintained at a higher level than in wild type by the lack of Cu-Zn-SOD. To test whether the increase in carotenoid accumulation is caused by intracellular ROS, the effect of antioxidants on light-inducible carotenoid synthesis was examined. We used N-acetyl-L-cysteine (37), 1,3-dimethylurea (38), and \(n\)-propyl gallate (39) as antioxidants. N-Acetyl-L-glycine, which has no antioxidative effect, was used as a control for the N-acetyl-L-cysteine. Each antioxidant was used at a final concentration of 1 mM. This concentration had no inhibitory effect on the mycelial growth (data not shown). The mycelia were treated with each reagent for 1 h in darkness and then were exposed to light for 2 h, and mycelial carotenoids were measured. The accumulation of carotenoids after antioxidant treatment followed by illumination was suppressed by about 40–50% compared with the control experiments in both wild type and the \textit{sod-1} mutant (Fig. 3A).

Next, we examined the effect of antioxidant on light-inducible gene expression in the \textit{sod-1} mutant. After co-incubation with N-acetyl-L-cysteine for 1 h, the mycelia were exposed to light for 1 h. Light-inducible accumulation of \textit{al-1} mRNA was determined by Northern blot analysis. The accumulation of \textit{al-1} mRNA in the mycelia treated with N-acetyl-L-cysteine was suppressed by about 40% of that observed in the sample without it (Fig. 3B).

The increases in the accumulation of \textit{al-1}, \textit{-2}, \textit{-3}, and \textit{bli-4} mRNAs in the \textit{sod-1} mutant may be dependent on the newly expressed \textit{uc-1} gene products after illumination (Fig. 2C). To test whether \textit{al-1} are under the control of \textit{de novo} synthesized WC-1, we examined the effect of cycloheximide inhibiting \textit{de novo} protein synthesis on the light-inducible accumulation of \textit{al-1} mRNA in wild type and the \textit{sod-1} mutant. After treatment with cycloheximide for 1 h, light-inducible accumulation of \textit{al-1} mRNA in the mycelia exposed to light for 15 min was assessed by Northern blot analysis. The increase in the accumulation of \textit{al-1} mRNA in the \textit{sod-1} mutant was not affected by the treatment with cycloheximide (Fig. 4). These results suggested that increases in the accumulation of light-inducible mRNAs in the \textit{sod-1} mutant do not require \textit{de novo} synthesis of a protein component such as WC-1 and VVD.

**Genetic Analysis of the Light-inducible Carotenoid Synthesis** Using the \textit{sod-1};\textit{uc-1}, \textit{sod-1} \textit{uc-2}, and \textit{sod-1};\textit{vvd} Double Mutants—The expression of the \textit{al-1}, \textit{-2}, and \textit{-3} genes is regulated downstream of the WC protein signaling cascade (12, 13). The \textit{sod-1} mutant showed a higher level of accumulation of light-inducible genes (Fig. 2). The intracellular ROS may act to stimulate carotenoid synthesis via WC proteins. To test this possibility, we observed the carotenoid synthesis in \textit{sod-1};\textit{uc-1} and \textit{sod-1} \textit{uc-2} double mutants compared with the \textit{uc-1} and \textit{uc-2} single mutants. These double mutants series, the \textit{sod-1}; \textit{uc-1}, \textit{uc-1} \textit{uc-2}, and \textit{sod-1};\textit{vvd} mutants, showed the lack of Cu, Zn SOD (Fig. 5A). In continuous darkness, wild type and the \textit{sod-1} mutant accumulated low levels of the carotenoids in mycelia compared with that of the \textit{uc-1} and \textit{uc-2} mutants (Fig. 5C). The double mutants, \textit{sod-1};\textit{uc-1} and \textit{sod-1} \textit{uc-2}, also failed to synthesize carotenoid after illumination (Fig. 5, B and C). These results indicate that the effects of ROS depended on the function of WC proteins.

Furthermore, a double mutant between \textit{sod-1} and \textit{vvd}, \textit{sod-
**FIG. 2.** Accumulation of light-inducible transcripts after illumination. A, Northern blot analysis of al-1, al-2, al-3, bli-4, and con-10 mRNA in wild type and the sod-1 mutant, and al-1 mRNA in the vvd mutant. After incubation of mycelia of the above strains for 36 h in darkness, total RNA was extracted from the mycelia after illumination (20 μE m⁻² s⁻¹) for 0.25, 0.5, 1, 2, and 3 h. Total RNA was separated on agarose gel, transferred onto a piece of membrane, and hybridized with ³²P-labeled probe. β-tub and 25 S rRNA were used for monitoring equal loading. B, densitometric analysis of the Northern blot analysis. The abundance of al-1, bli-4, and con-10 mRNA was calculated relative to β-tub mRNA, and that of al-2 and al-3 was 25 S rRNA. Relative values were calculated using the values in darkness for the wild type. C, wc-1 mRNA in wild type, the sod-1 and vvd mutant after illumination for 30 min was quantified by RT-PCR. The amplified wc-1 was detected by Southern blot analysis. β-tub was used as a loading control. The fragment amplified by RT-PCR was separated on agarose gel and stained with ethidium bromide. Each value is the average of three independent experiments with standard errors.

**FIG. 3.** Light-inducible carotenoid and al-1 mRNA accumulation after treatment with antioxidants. A, after mycelia were treated in the presence of various antioxidants (1 mM each of N-acetyl-l-cysteine, 1,3-dimethylurea, or n-propyl gallate), they were exposed to light (20 μE m⁻² s⁻¹) for 2 h. Carotenoid accumulation per g dry weight of mycelia was quantified by A₄₇₀ nm. N-Acetylglycine was used as a negative control. B, the accumulation of al-1 mRNA in wild type and the sod-1 mutant treated with 1 mM N-acetyl-l-cysteine after illumination (20 μE m⁻² s⁻¹) for 1 h. Each value is the average of three independent experiments with standard errors.
and under light (20 \text{ min}) of carotenoid in the mycelia in darkness compared with that of the vvd mutant after 2 h of illumination. This result suggests that there may be a parallel relationship between sod-1 and vvd in the pathway to induce the carotenoid synthesis.

Light-inducible Perithecial Polarity in the sod-1 Mutant—We next investigated a light-responsive phenotype other than carotenoid synthesis in the sod-1 mutant. Previously, we found that the perithecial polarity was controlled by illumination, because the perithecial beaks corresponding to 80–90% of total perithecia are formed upward of perithecia under illumination in the wild type of N. crassa, while, in constant darkness, perithecial beaks formed upward was reduced to about 40% of total perithecia (6, 7) (Fig. 6). The effects of illumination on the probability of beaks formed at the top of the perithecia were determined in the mutants used above. In the sod-1 mutant, the upward positioning of the beaks was 20% of total perithecia both in darkness and under illumination, which was the same level as observed in the ndk-1P72H mutant, whereas phototropism of perithecial beaks toward directional light was normal in both sod-1 and ndk-1P72H mutants (Fig. 6A). In the photograph of perithecia under light in Fig. 6A, the positioning of beaks in wild type was upward. However, the beaks bent toward the directional light showing positive phototropism, because the light was provided from the left side in the photographs. On the other hand, the positioning of beaks in the sod-1 mutant was mostly sideward, whereas the beaks bent toward the left side. The upward positioning of the beaks in the double mutant between sod-1 and ndk-1P72H was 10% of total perithecia both in darkness and under illumination, and an additive effect on perithecial polarity could be detected (Fig. 6B). This result suggests that there may be a parallel relationship between sod-1 and ndk-1 in the pathway to induce perithecial polarity irrespective of illumination. Furthermore, the sod-1:vvd double mutant displayed deficient light response to induce perithecial polarity, similar to that observed in the sod-1 mutant, although the perithecial polarity in the vvd mutant under light was the same level as that observed in the wild type (Fig. 6B). This result indicates that the phenotype of the sod-1 mutant was not affected by the vvd mutation.

To examine the relation of sod-1 and wc-1 or wc-2, we examined the light-inducible perithecial polarity in the wc-1 wc-2, sod-1 wc-1, sod-1 wc-2, vvd, and sod-1:vvd mutants. In the wc-1 and wc-2 mutants, perithecia formed upward were 5% of total perithecia, respectively, irrespective of illumination, whereas the phototropism of perithecial beaks was not detected (Fig. 6A). The sod-1 wc-1 and sod-1 wc-2 double mutants showed neither significant light-induced perithecial polarity nor positive photo-
tropism of perithecial beak, similar to the wc single mutants (Fig. 6B). These results indicate that perithecial polarity in the sod-1 mutant is under the control of WC proteins irrespective of illumination, which was essentially the same result as observed in the carotenoid synthesis.

**DISCUSSION**

**Light-inducible Carotenogenesis Is Controlled by Intracellular ROS**—In this study, the sod-1 mutant showed accelerated light-induced carotenogenesis in the mycelia (Fig. 1). Although this phenotype in the sod-1 mutant was very similar to that of the vvd mutant, the sod-1 mutation was more effective on the initial rate (after 3 h of illumination) of the carotenogenesis than the vvd mutation (Fig. 1). This induction of carotenoid synthesis was suppressed by the exogenic application of antioxidants (Fig. 3). As a general role, SOD reduces levels of the superoxide anion radical $O_2^-$ that is generated by aerobic environments and aerobic metabolism, catalyzing conversion of $O_2^-$ to hydrogen peroxide, $H_2O_2$, and thus protecting the organism from oxidative toxicity (45). *N. crassa* has two types of SODs, Cu,Zn-SOD and Mn-SOD. The intracellular localization of Cu,Zn-SOD is cytosolic, whereas that of Mn-SOD is mitochondrial (35, 46). The sod-1 mutant used in this study lacked the cytosolic type of Cu,Zn-SOD. In plant, it has been known that in coleoptile tips, the intracellular ROS concentration rises predictably in the early light induction of carotenoid synthesis. Recently, in *N. crassa*, catalase-3-deficient mutant showed enhanced production of carotenoid synthesis in colonies under illumination (48). These results suggested that intracellular ROS affect light inducible carotenoid synthesis.

In Northern blot analysis, the light-inducible mRNAs including those for enzymes involved in the synthesis of carotenoids showed a sustained expression in the sod-1 mutant, when compared with the transient expression in the wild type (Fig. 2). Interestingly, the expression of con-10 was not significantly different compared with that of wild type (Fig. 2). By microarray analysis, the expression of light-inducible genes was classified in several patterns (49). It is suggested that the effects of ROS on the gene expression could also be classified to the different patterns in light responses.

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The accumulation of the al-1 transcript in the sod-1 mutant was reduced by treatment with antioxidants (Fig. 3B). These results suggest that intracellular ROS affect the expression of light-inducible genes.

In addition to al-1, -2, -3, and bli-4, the light-induced accumulation of the wc-1 transcript also showed an increase in the sod-1 mutant (Fig. 2). The sustained expression of these light-inducible genes in the sod-1 mutant may be caused by the increase in WC-1 protein after illumination, a critical blue light signal-transducing factor in the light-induced carotenoid synthesis in the mycelia (30). However, as the increase of al-1 mRNA in the sod-1 mutant was not affected even after treatment with cycloheximide (Fig. 4), light-inducible transcription in the sod-1 mutant must not require the de novo synthesis of WC-1 and VVD protein. Moreover, as the sod-1 wc-1 and sod-1 wc-2 double mutants failed to generate carotenoid after illumination (Fig. 5), suggesting that intracellular ROS could affect the initial stage in the light signal transduction pathway via WC proteins, independent from de novo protein synthesis.

The sod-1 and vvd mutants showed different kinetics in light-induced carotenoid synthesis (Fig. 1B). These results suggest that the effect in the process of ROS was different from...
that of VVD. Furthermore, the vvd mutant also showed sustained expression of al-1 mRNA under the light (44) (Fig. 2, A and B). However, the maximum accumulation of al-1 mRNA in the vvd mutant was observed at 1 h, and that in the sod-1 mutant was at 15 min after illumination (Fig. 2, A and B). WC-1/WC-2 promoted the vvd expression after illumination, and the vvd product suppressed the WC-1/WC-2 function (28, 50). VVD may function about 1 h after illumination. Hence, the sod-1 mutation caused al-1 accumulation at 15 min after illumination, ROS could function within 15 min and then VVD may function. The sod-1 vvd double mutant showed an additive effect on light-induced carotenoid synthesis (Fig. 6B).

This additive phenotype could be caused by ROS induced by the sod-1 mutation in the initial stage and de novo synthesized VVD may function at around 1 h after illumination. How does ROS control the expression of light-inducible genes? Two possibilities exist, regulation at the transcriptional level and regulation at the post-transcriptional level. If the regulation is at the transcriptional level, ROS would act directly on WC-1 and WC-2 proteins via some kind of modification such as phosphorylation of WC-1 and binding between WC-1 and WC-2. WC-1/WC-2 bound to the cis-acting element (light-responsive elements) in the frequency (frq) promoter. The mobility shift assay showed a decrease in the bound WC-1/WC-2 to the promoters by illumination (51). The WC-1/WC-2 binding to light-responsive elements may be controlled by ROS.

The LOV domains in Phototropin are thought to function as internal sensors of oxygen, redox potential, and light (52, 53). Flavin-binding proteins such as cryptochromes and Phototropins may be affected by the changes in redox state. For example, ROS may control the reversibility of the adduct formation between the chromophore and cysteine residue, which could be modified by ROS, within the LOV domain (54). Hence, intracellular ROS may change the redox state of the WC-1 protein and regulate enzymatic activities such as DNA binding and transcriptional activities.

As another possibility, light-inducible genes may be controlled by ROS at the post-transcriptional level. In general, the stability of transcripts is regulated by RNA-binding proteins or RNases (55). In Synecocystis, psbA mRNA stability is determined by the intracellular redox state (56, 57). These reports suggested that factors other than light have the capacity to affect the function of WC-1/WC-2. Hence ROS endogenously generated by mitochondria and intracellular metabolisms may affect the perithecial polarity under continuous darkness via WC-1/WC-2.

Another question was how the phenotypes of the sod-1 and ndk-1P72H mutants could be additive in the double mutant irrespective of illumination (Fig. 6B)? Recently, we found that NDK-1 regulated catalase activity in N. crassa. The primary function of catalase is to reduce hydrogen peroxide to water. Intracellular concentration of hydrogen peroxide could be increased in the ndk-1P72H mutant. The ROS distribution may be disturbed by the increase of hydrogen peroxide. Consequently, light-inducible perithecial polarity in the ndk-1P72H mutant may be disrupted. The increase of superoxide and hydrogen peroxide in the sod-1 and ndk-1P72H mutant may lead to severer (additive) defects in light-inducible perithecial polarity irrespective of illumination than the single mutant of ndk-1P72H or sod-1.

The ROS are suggested to function as signaling messengers in various biological phenomena in a wide range of organisms (62, 63). In this study, we propose that intracellular ROS may function as a novel light signal transducer in carotenogenesis and perithecial polarity.

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