

A LexA-related protein regulates redox-sensitive expression of the cyanobacterial RNA helicase, *crhR*

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ABSTRACT

Expression of the cyanobacterial DEAD-box RNA helicase, *crhR*, is regulated in response to conditions, which elicit reduction of the photosynthetic electron transport chain. A combination of electrophoretic mobility shift assay (EMSA), DNA affinity chromatography and mass spectrometry identified that a LexA-related protein binds specifically to the *crhR* gene. Transcript analysis indicates that *lexA* and *crhR* are divergently expressed, with *lexA* and *crhR* transcripts accumulating differentially under conditions, which respectively oxidize and reduce the electron transport chain. In addition, expression of the *Synechocystis* *lexA* gene is not DNA damage inducible and its amino acid sequence lacks two of three residues required for activity of prototypical LexA proteins, which repress expression of DNA repair genes in a range of prokaryotes. A direct effect of recombinant LexA protein on *crhR* expression was confirmed from the observation that LexA reduces *crhR* expression in a linear manner in an *in vitro* transcription/translation assay. The results indicate that the *Synechocystis* LexA-related protein functions as a regulator of redox-responsive *crhR* gene expression, and not DNA damage repair genes.

INTRODUCTION

The ability to adapt to a dynamic light environment is crucial for the survival of photosynthetic organisms and includes both short- and long-term responses. Light sensing occurs either via direct mechanisms involving photoreceptor proteins, or indirectly through light-driven changes in the redox status of the electron transport chain between Q_A in photosystem II and Q_O in cytochrome b_6f (1–4). Electron carriers in this region of the inter-photosystem electron transport chain perform essential roles in redox-sensing in higher plant chloroplasts, regulating expression of nuclear- and chloroplast-encoded genes involved in photosynthesis (5–9). For example, a direct link between the redox poise

of plastoquinone and chloroplast gene expression has been shown for the *psbA* and *psaAB* genes, allowing rapid cellular response to the light environment via sensing of the redox status of the electron transport chain (7). In contrast, the factors responsible for transduction of the electron transport redox poise to transcription regulation remain poorly characterized. Possible transducers identified in spinach chloroplasts include an unidentified 31 kDa dimeric protein shown to bind the *psaAB* promoter (10) and the TSP9 thylakoid-associated protein. Redox-mediated phosphorylation of TSP9 on three threonine residues releases the protein from the thylakoid membrane potentially allowing it to play a role as a signaling factor responsible for transducing plastoquinone redox poise to gene expression (11).

In prokaryotic cyanobacteria, the redox status of the electron transport chain carriers also regulates expression of a limited number of photosynthetic and non-photosynthetic genes. Expression of the RNA helicase, *crhR* (1), glutamine synthetase, *glnA* (12), PII protein, *glnB* (13), α and β subunits of phycocyanin, *cpcBA* (14), photosystem proteins (14–16) and a transcriptional regulator (17) has been attributed to the redox poise of plastoquinone and/or cytochrome b_6f . Members of the signal transduction pathway(s) associated with sensing and transducing changes in redox status to the transcriptional machinery also remain to be identified in cyanobacteria. Proposed mechanisms in cyanobacteria include a redox-responsive two-component signal transduction pathway (16) and the redox-sensitive transcriptional regulators, NtcA and NblS (17,18). A potential sensor is the membrane-associated sensor histidine kinase NblS, which responds and controls photosynthesis-related gene expression in response to high light and nutrient stress (18). NblS contains a redox-sensing PAS domain potentially involved in the sensing and transduction of high light/nutrient stress induced changes in photosynthetic or cellular redox poise to as yet uncharacterized transcriptional regulators (18). While a number of redox-sensitive transcriptional regulators have been described in prokaryotic systems, the physiological electron donors have not been identified (19–23). NtcA is one transcriptional regulator whose activity has been shown to involve a complex interaction between cellular nitrogen levels, thiol group redox poise and photosynthetic electron flow although not directly correlated with the redox state of the plastoquinone pool (17). NtcA controls expression of

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genes involved in nitrogen acquisition, repressing *gifA* and *gifB* and activating expression of *glnA*, *glnN* and *glnB* in response to both nitrogen levels and electron transport (24).

Expression of the *Synechocystis* DEAD-box RNA helicase, *crhR* (Cyanobacterial RNA Helicase-Redox), is also regulated by the redox poise of the electron transport chain (1). *crhR* transcripts accumulate when the electron transport chain is reduced, either from light-driven electron flow or respiratory electron flow generated by the metabolism of exogenously supplied glucose. In contrast, a reduction in electron flow, leading to oxidation of the electron transport chain, decreases *crhR* transcript accumulation. These results are corroborated by results obtained using electron transport inhibitors or alteration of light quality which alter *crhR* induction, confirming redox-regulated expression and identifying the redox poise of the electron transport chain between Q_A in photosystem II and Q_O in the cytochrome *b₆f* complex, as the potential sensor for redox-dependent regulation (1). Biochemically, CrhR exhibits enzymatic activities characteristic of RNA helicases, including RNA-dependent ATPase activity and ATP-stimulated RNA unwinding (25). In addition, CrhR also possesses ATP-dependent RNA annealing activity (26). Thus, CrhR has been proposed to regulate gene expression at the translational level through its ability to rearrange RNA secondary structures of RNA substrates, potentially of other redox-regulated gene transcripts (1,26).

As an initial step to elucidate upstream factors involved in the redox-regulated expression of the *crhR* gene, we have identified a LexA-related protein that controls *crhR* transcript accumulation. Treatments known to enhance *crhR* transcript accumulation reduce *lexA* levels and vice versa. A direct effect of the recombinant His-tagged LexA protein (rLexA) on *crhR* expression was confirmed from the observation that rLexA reduces *crhR* expression in an *in vitro* transcription/translation assay. LexA thus appears to function as a repressor of *crhR* transcription when *crhR* is not required i.e. under conditions which oxidize the electron transport chain. In concurrence with this conclusion, expression of the *Synechocystis* *lexA* gene is not DNA damage inducible and its amino acid sequence lacks two of three residues required for the self-cleavage activity of prototypical LexA proteins (27). We discuss the potential significance of a LexA-related repressor in the regulation of redox-responsive gene expression and, consequently, the implications of this novel role performed by LexA in *Synechocystis*.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Synechocystis sp. strain PCC 6803 was maintained at 30°C on BG-11 agar (28) solidified with 1% (w/v) Bacto-Agar (Difco Laboratories, Detroit, MI) and grown photoautotrophically at 30°C under continuous illumination at a constant intensity of 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Liquid BG-11 cultures were aerated by shaking at 150 r.p.m. and continuous bubbling with humidified air. Dark conditions were created by wrapping the flasks in aluminum foil. Glucose (5 mM) was added where indicated. To induce DNA damage, cells were exposed to short-wave ultraviolet light (UV-C; 254 nm) at a dose rate of 150, 300 or 600 J/m^2 using a

XL-1000 UV crosslinker (Spectronics Corporation) and subsequently incubated in the dark for 1 h prior to harvesting.

Escherichia coli strains DH5 α and JM109 were used for propagation and protein expression of plasmid constructs, respectively. Cultures were grown in Luria-Bertani (LB) medium at 37°C and aerated by shaking at 200 r.p.m. Ampicillin (100 $\mu\text{g/ml}$) was added where appropriate.

Plasmid constructs

A deletion series within the *crhR* promoter/open reading frame (ORF) was created from a 3 kb EcoRI fragment encompassing the *crhR* promoter, 5'-untranslated region (5'-UTR) and the *crhR* ORF (1). Plasmid DNA was digested with NotI and SacI, and a deletion series produced using the Erase-A-Base Kit (Promega) according to the manufacturer's instructions. Two additional deletion constructs were created by restriction enzyme digestion. SpeI removed a 328 bp fragment to construct KC+125. The KC+219 construct was created by EcoRI / XmnI digestion to liberate a 2.6 kb fragment containing the *crhR* ORF downstream of +219 but lacking the promoter region. This fragment was blunt end ligated into EcoRV digested pBluescript KS+ (Stratagene).

The *lexA* gene was amplified by PCR to generate an in frame translational fusion in the pRSETB plasmid vector (Invitrogen). The *lexA* insert DNA was generated using primers LPF-27 (5'-ACTGGTGGATCCGAACCTCTCACCCGAGCC-3') and LPF-2 (5'-GAAACAAAAGCTTAGGACG-3') and *Synechocystis* chromosomal DNA as template. PCR were performed in a volume of 50 μl , containing 300 nM primer and 0.8 U of Expand High Fidelity enzyme mix (Roche) according to the manufacturer's instructions. The PCR program consists of 30 cycles of 1 min denaturation at 95°C, 30 s annealing at 55°C and 1 min extension at 68°C; and terminated with 4 min extension at 68°C. Purified DNA was digested with BamHI and HindIII (restriction sites underlined) and cloned into BglII and HindIII digested plasmid DNA. The resulting plasmid pLexA expresses a recombinant LexA polypeptide (rLexA) containing a 37 amino acid residue N-terminal HIS tag. pLexA was sequenced to confirm successful in frame insertion of the *lexA* gene.

Generation of promoter fragments

Promoter fragments were PCR amplified from the deletion plasmids using the M13 forward primer (5'-GTAAACGACGGCCAGT-3') and GWO-45 (5'-AAGCCAATGTGCGCCAAGAG-3') (Figure 1A). PCR were performed as described above using an annealing temperature of 45°C. BssHII / BssSI digestion was used to generate fragments corresponding to KC+125 and KC+219. DNA fragments were purified from 1 \times TAE agarose gels using GENECLEAN[®] II (BIO 101).

DNA affinity column purification

To purify proteins binding to the *crhR* promoter region, μMACS Streptavidin magnetic separation was performed (Miltenyi Biotec). *Synechocystis* cultures (300 ml) were harvested at mid-log phase and resuspended in 2 ml cyanobacterial protein extract buffer [20 mM Tris-HCl (pH 8), 10 mM NaCl, 1 mM EDTA (pH 8) and 5 mM DTT] containing protease inhibitors (Complete Mini Protease Inhibitor Cocktail,

Northern analysis

Total RNA was isolated from *Synechocystis* by mechanical lysis, separated on a 1.2% formaldehyde gel, and transferred to a nylon membrane (Hybond N⁺) as described previously (29). Blots were hybridized overnight at 65°C with the appropriate probe in aqueous buffer (5× SSPE, 5× Denhardt's, 0.5% SDS) and washed for 10 min at 65°C once in 1× SSPE, 0.1% SDS and once in 0.1× SSPE, 0.1% SDS. *lexA* and *crhR* DNA fragments were randomly labeled with [α -³²P]dCTP using random hexanucleotide primers (Roche). The probes correspond to: *lexA*, a 750 bp BglII / HindIII fragment encompassing the entire ORF; *crhR*, a 784 bp internal BstEII fragment; and *recA*, a 1091 bp riboprobe encompassing the ORF labeled with [α -³²P]UTP (Promega). Membranes were stripped by incubation in boiling 0.1% SDS and probed with the *Synechocystis rnaseP* gene as a control for RNA loading (30).

In vitro transcription/translation assays

In vitro transcription and translation assays were performed using the Promega *E.coli* S30 extract system for circular DNA in a final reaction volume of 25 μ l. The plasmids pCrhR (IV) and pWM3-2 (29) were used for *in vitro* expression of the *crhR* and *crhC* genes, respectively. pCrhR (IV) was prepared by ligating a 2.2 kb BamHI / EcoRI fragment of CS0096-9 (1) into pBluescript KS+ to remove downstream sequences encoding the *argC* gene. The pCrhR (IV) and pWM3-2 plasmids contain 2.2 and 2.4 kb inserts, respectively encoding the promoters, ORFs, and 5'- and 3'-UTRs of *crhR* and *crhC*, respectively. Reactions were performed according to manufacturer's instructions using 1 μ g plasmid DNA, corresponding to 0.29 and 0.28 pmol DNA for pCrhR (IV) and pWM3-2, respectively. Reaction products were separated on a 10% (w/v) polyacrylamide gel and subjected to autoradiography. Binding reactions containing rLexA were performed according to manufacturer's instructions with an initial 5 min incubation to allow protein-binding to the *crhR* gene. Control reactions were performed to determine the effect of rLexA on *crhR* expression in the presence of BSA and expression of an unrelated RNA helicase, *crhC* (30), from its own promoter.

RESULTS

Promoter deletion series delineates the protein-binding site within the *crhR* gene

EMSA assays were performed using nine plasmid constructs containing deletions of the *crhR* promoter (Figure 1A) to delineate the protein-binding region. Intact *crhR* promoter (KC-179) and deletions up to position +77 of the *crhR* transcript (KC+77) exhibited decreased mobility on a native PAGE gel upon incubation with *Synechocystis* protein extract (Figure 1B, lanes 1–14). The KC+125 DNA target, deleted to +125 of the transcript, exhibited a reduced amount of shift (Figure 1B, lanes 15 and 16), while deletion to +219 completely abolished the mobility shift (Figure 1B, lanes 17 and 18). Together, these results indicate that the protein-binding site is located downstream of the translational start codon (+110) in the region of DNA surrounding an SpeI site (+125). Sequence specificity of binding was shown by

competition assays in the presence of increasing amounts of either specific or non-specific competitor DNA (Figure 1C). Addition of specific competitor DNA (KC-179) progressively challenged formation of the shifted complex (Figure 1C, Specific competitor). Conversely, inclusion of a similar sized fragment containing the pBluescript KS⁺ multiple cloning site had no effect on the mobility shift (Figure 1C, non-specific competitor). Taken together, these results indicate that at least one soluble *Synechocystis* protein interacts with the *crhR* gene in a sequence-specific manner.

Synechocystis LexA-related protein binds within the *crhR* ORF

To identify the protein responsible for altered mobility of the *crhR* gene, DNA affinity column chromatography was performed using light-grown *Synechocystis* soluble protein extracts and biotinylated KC+5 DNA (239 bp). A single polypeptide with an apparent molecular weight of 28 kDa was recovered in the high stringency 1 M KCl elution (Figure 2A). The single significant hit (score 92) identified by in-gel tryptic digestion and LC/MS/MS corresponded to the *Synechocystis* gene sl11626, which has been annotated as encoding the transcriptional repressor LexA (<http://www.kazusa.or.jp/cyanobase/>). Analysis of the deduced *Synechocystis* LexA amino acid sequence revealed that the sequence lacks the Ala-Gly self-cleavage site and the serine of the Ser-Lys dyad active site present in *E.coli* LexA, both of which are required for LexA self-cleavage [Figure 2B; (31)]. Furthermore, an SOS-like box, similar to those identified as LexA binding sites in *E.coli* (32,33), *Bacillus subtilis* (34) and *Mycobacterium tuberculosis* (35) could not be identified within the upstream sequence of either *lexA* or *crhR* (data not shown). However, a sequence related to the putative cyanobacterial SOS box (36), matching 7 of 9 essential residues with required spacing between essential residues, was identified within the protein-binding domain in *crhR* (Figure 2C). This sequence includes the SpeI site, possibly explaining the reduced shift observed with the SpeI generated KC+125 fragment (Figure 1B).

Synechocystis *lexA*, *crhR* and *recA* transcript accumulation

crhR transcript accumulation is regulated by the redox poise of the plastoquinone pool with treatments leading to reduction of plastoquinone correlating with an increase in *crhR* transcript accumulation, whereas conditions that lead to the oxidation of the plastoquinone pool result in decreased *crhR* accumulation (1). Northern analysis was therefore performed to determine the relationship between *lexA* and *crhR* transcript accumulation under varying redox conditions (Figure 3A). Growth in the light (Figure 3A, lane 1), conditions favoring *crhR* transcript accumulation, correlate with reduced levels of *lexA* transcript. Conversely, growth in the dark (Figure 3A, lane 2) reduces *crhR* while enhancing *lexA* transcript accumulation. The addition of glucose (5 mM) to light-grown cells enhanced *crhR* and *lexA* transcript accumulation (Figure 3A, lane 3). *crhR* expression was significantly induced in response to cold stress (20°C; Figure 3A, lane 4), concomitant with the complete repression of *lexA* transcript accumulation. The data indicates

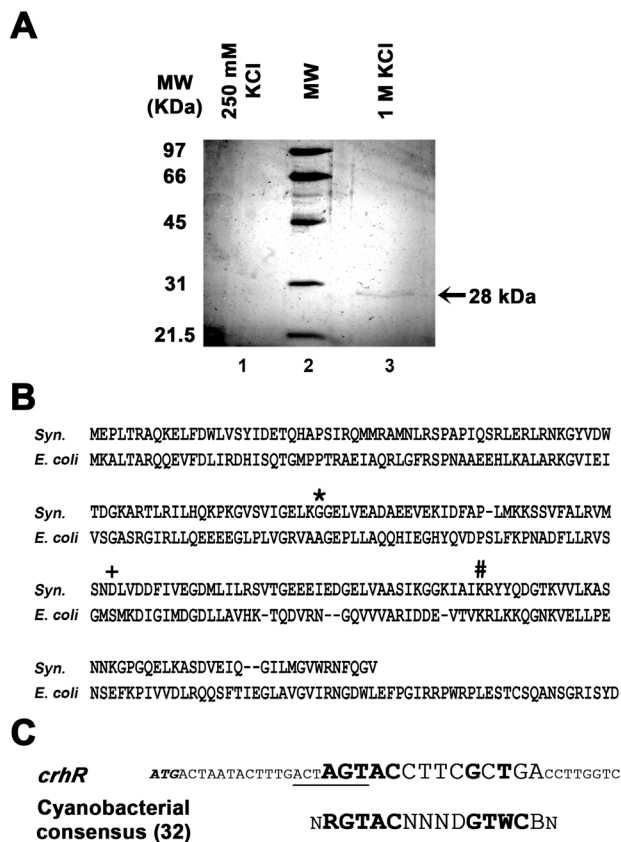


Figure 2. Isolation and characterization of a *crhR* regulatory protein by affinity chromatography and LC/MS/MS. (A) A 28 kDa polypeptide interacts with the *crhR* ORF. A single polypeptide was isolated by DNA affinity chromatography using KC+5 as the target. Non-specifically bound proteins were removed by increasing KCl washes. Silver staining of eluted proteins separated by a 10% SDS-PAGE reveals a single polypeptide in the 1 M KCl elution. LC/MS/MS identified this polypeptide as the *Synechocystis* gene annotated as encoding LexA. Lane 1, 250 mM KCl wash; lane 2, low molecular weight standards (BioRad); lane 3, 1 M KCl wash. (B) Amino acid sequence analysis of the *Synechocystis* (gray) and *E. coli* (black) LexA proteins. Residues essential for *E. coli* LexA self-cleavage in response to DNA damage are indicated as follows: * = Ala-Gly self-cleavage site; + = Ser and # = Lys indicate the Ser-Lys dyad active site. (C) Alignment of the putative LexA binding region of the *crhR* gene (SpeI site is underlined) with the consensus cyanobacterial LexA binding sequence (32). Conserved residues are bolded. The LexA binding sequence within the *crhR* matches at 7 of 9 conserved residues with appropriate spacing.

differential regulation of *crhR* and *lexA* expression in response to alterations in the redox status of the electron transport chain, implying that LexA functions as a regulator of *crhR* expression.

LexA association with the *E. coli* SOS response and repression of DNA damage repair gene expression warranted comparative analysis of *Synechocystis* *lexA*, *recA* and *crhR* expression in response to DNA damage. DNA damage was induced by UV-irradiation, and the resulting expression patterns were examined by northern analysis (Figure 3B). In contrast to *E. coli*, following UV-irradiation neither *Synechocystis* *lexA* nor *recA* expression (Figure 3B, lanes 2–4) was induced above basal levels detected in dark grown cells (Figure 3B, lane 1). In fact, in contrast to other prokaryotic systems, *recA* was expressed at very low levels

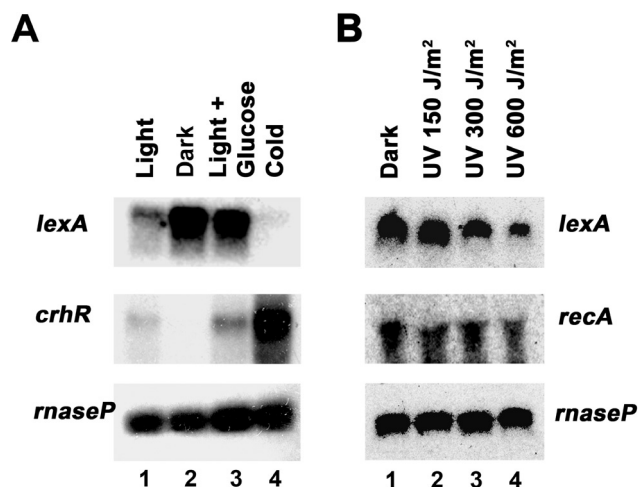


Figure 3. Transcript analysis. Total RNA (30 μ g) was isolated from *Synechocystis* cells grown as indicated. RNA was separated on a 1.2% formaldehyde agarose gel, transferred to Hybond N⁺ and hybridized with the indicated ³²P-labeled probe. (A) *lexA*, *crhR* and *rnaseP* transcript accumulation following incubation in the light. Lane 1, 3 h light; lane 2, 3 h dark; lane 3, light plus 5 mM glucose; lane 4, cold stress for 3 h (20°C). (B) *recA*, *lexA* and *rnaseP* transcript accumulation in response to increasing levels of UV-irradiation. Lane 1, 1 h dark; lane 2, UV irradiated with 150; lane 3, 300; lane 4, 600 J/m² followed by a 1 h incubation in the dark.

under all conditions tested, requiring riboprobe detection and extended exposure times. *lexA* transcript accumulation was also not altered by DNA damage-induced by mitomycin C (data not shown). Similarly, expression of *crhR* was not UV-inducible (data not shown); rather, it followed the expected decrease in transcription that occurs in wild-type cells in the dark (1). The lack of induction of the *Synechocystis* *recA*, *lexA* and *crhR* genes following DNA damage suggests these gene products are not required during the cellular response to DNA damage.

Synechocystis LexA interacts with the *crhR* gene

Recombinant His-tagged LexA (rLexA) was purified to near homogeneity and used to test interaction with the KC+5 *crhR* promoter fragment (Figure 4). KC+5 *crhR* promoter DNA mobility was reduced by incubation with total *Synechocystis* protein extracts (Figure 4A, lane 8 versus lane 1). Mobility of the KC+5 DNA target was also altered by incubation with purified rLexA, with alteration of target DNA mobility exhibiting rLexA concentration dependence (Figure 4A, lanes 2–7). *E. coli* soluble protein extracts did not alter *crhR* DNA target mobility, indicating that *E. coli* proteins do not bind the *crhR* gene (Figure 4A, lane 9). Sequence-specific binding was demonstrated by competition assays in the presence of increasing concentrations of either specific or non-specific competitor DNA. Addition of unlabeled specific competitor (KC+5) challenged formation of the shifted complex at all concentrations tested (Figure 4B, lanes 2–5), with addition of ≥ 50 -fold excess of unlabeled target abolishing shift of the DNA target. In contrast, incubation with non-specific competitor DNA, an internal *lexA* fragment similar in size to the specific competitor, did not significantly alter mobility shift at comparable concentrations (Figure 4B,

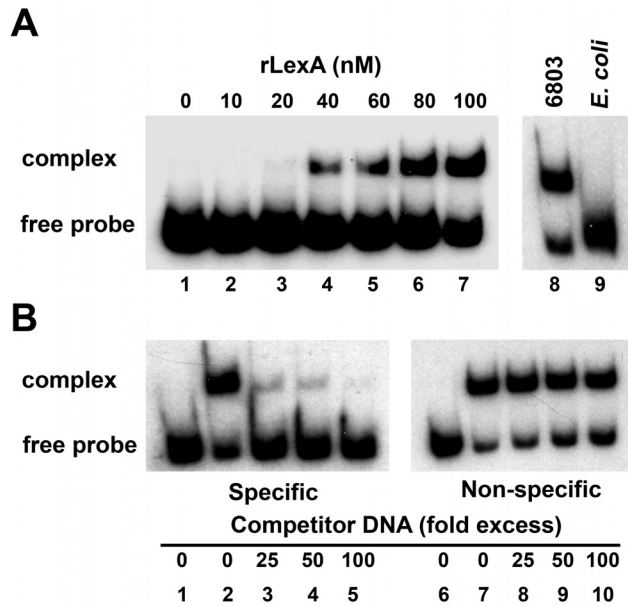


Figure 4. LexA-related protein-binding analysis. EMSA using recombinant LexA (rLexA) were performed to confirm interactions between LexA and the *crhR* gene. (A) rLexA concentration curve. Increasing concentrations of rLexA were incubated with ³²P-labeled KC+5. As controls, rLexA was also incubated with *Synechocystis* (lane 8) and *E.coli* (lane 9) soluble protein extracts. (B) DNA competition assays. rLexA (100 nM) was incubated with ³²P-labeled KC+5 and the indicated fold excess of either specific competitor DNA (unlabeled KC+5; lanes 1–5) or non-specific competitor DNA (internal *lexA* fragment; lanes 6–10).

lanes 7–10). Together, the results indicate that recombinant LexA interacts with the *crhR* gene in a sequence-specific manner.

LexA represses *crhR* gene expression *in vitro*

An *in vitro* transcription and translation system was used to confirm LexA regulation of *crhR* gene expression from its native promoter. As shown in Figure 5A, CrhR protein accumulation decreased in response to increasing rLexA concentration. Quantification of these results indicated that the rLexA inhibition of CrhR expression was linear with respect to rLexA concentration (Figure 5B). The specificity of repression was demonstrated by the lack of change in the levels of the plasmid-encoded β-lactamase protein, a non-LexA regulated protein. Similarly, *crhR* expression was unaffected by incubation in the presence of 1 pmol BSA (Figure 5C), a protein concentration at which rLexA significantly altered *crhR* expression. Furthermore, *in vitro* transcription and translation of a second cyanobacterial RNA helicase, *crhC* (29,30), was also unaffected by 1 pmol rLexA (Figure 5C). Together, these results indicate that LexA specifically regulates *crhR* transcription in a negative fashion.

DISCUSSION

We have begun investigation of the signal transduction pathway transducing the redox poise of the electron transport system to the transcription apparatus in cyanobacteria. In this

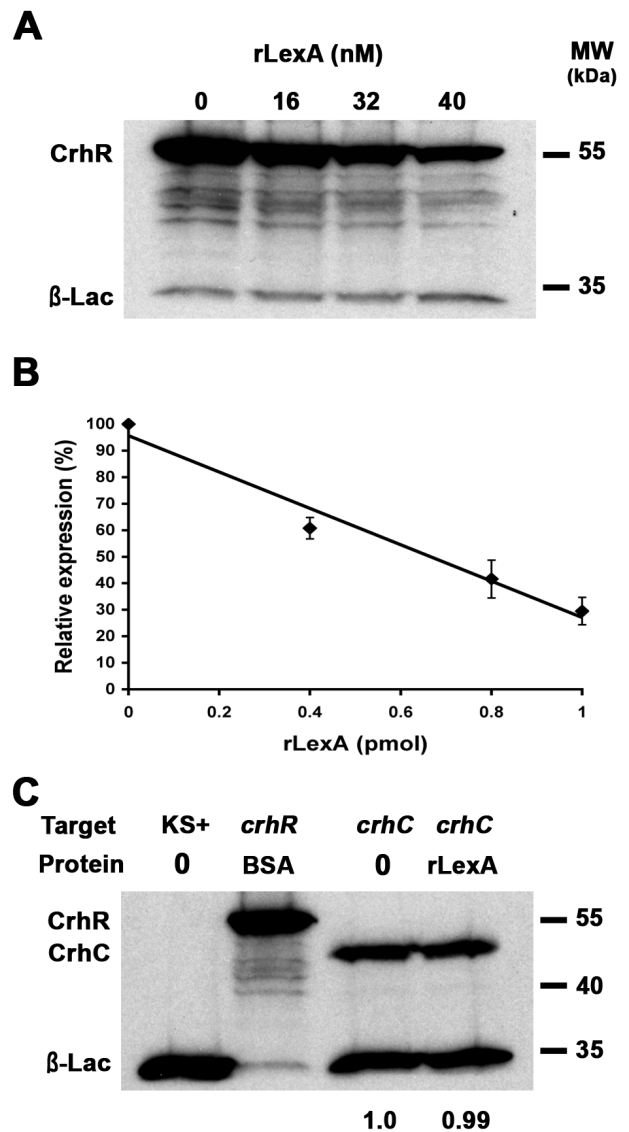


Figure 5. *In vitro* transcription/translation. *In vitro* reactions were used to investigate the nature of the LexA regulatory relationship with the *crhR* gene. (A) CrhR protein (55 kDa) accumulation in the presence of increasing rLexA concentration. A plasmid (pCrhR IV) containing the complete *crhR* gene, including 289 bp upstream of the translation start, was incubated in a transcription/translation mixture in the presence of increasing rLexA (0–40 nM). (B) Quantification of rLexA effect on CrhR expression. CrhR expression in the presence of increasing rLexA concentration was quantified from triplicate, independent replicates similar to the data shown in (A) using ImageQuant (Molecular Dynamics). Standard deviations from the means are shown. (C) Specificity of rLexA regulation. As control reactions, the transcription/translation efficiency of CrhR was evaluated in the presence of a non-specific protein, BSA (1 pmol). In addition, accumulation of the temperature-regulated cyanobacterial RNA helicase, CrhC (24,25) was determined in the presence and absence of rLexA (1 pmol). The relative level of CrhC expression is shown below the figure, as determined using ImageQuant.

paper we show that a LexA-related protein regulates expression of the redox-responsive RNA helicase, *crhR*. This identification implies a novel function for LexA in *Synechocystis*, a conclusion consistent with previous studies suggesting that LexA may regulate expression of carbon metabolism

and bidirectional hydrogenase genes in *Synechocystis* (37–39).

The LexA binding site is located downstream of the *crhR* transcription start site, requiring sequences surrounding +125 of the *crhR* transcript. This localization suggests a regulatory mechanism for the *Synechocystis* LexA-related protein that differs from LexA regulation of DNA damage inducible genes in *E.coli* and other bacteria, where the LexA binding site (SOS box) surrounds the transcriptional start (32,33). Similar regulatory element arrangements, where transcription factors bind downstream of the transcription start site, have been observed in the cyanobacterium *Synechococcus* sp. strain PCC 7942 (40). In fact, the light responsive transcription of the *psbA* and *psbD* gene families in *Synechococcus* requires enhancer elements located downstream of the transcription start (40–42). The LexA DNA binding site within the *crhR* ORF is therefore consistent with regulatory protein-binding sites localized in other genes whose expression is known to be regulated by either light or redox signals. Unfortunately, the DNA binding proteins interacting with these other sites remain to be identified.

A combination of DNA affinity chromatography and mass spectrometry identified the protein interacting with the *crhR* gene as being related to LexA. Northern blot analysis showed that *Synechocystis* LexA transcripts accumulate when cells are grown under conditions correlating with the repression of *crhR* accumulation. Based on these results, it appears that LexA functions as a negative regulator of *crhR* expression. Negative regulation was confirmed using an *in vitro* transcription/translation assay, which demonstrated that LexA binding interferes with *crhR* expression possibly through interference with promoter recognition and/or transcription initiation. Based on these results, it appears that *Synechocystis* LexA functions as a repressor of *crhR* expression. LexA activity is well studied in *E.coli* and other prokaryotes where it regulates expression of ~20 unlinked genes associated with DNA damage repair, the SOS regulon, which include *recA* and *lexA* (27). Derepression occurs following DNA damage, and requires RecA-stimulated LexA autocleavage and subsequent derepression of *lexA*, *recA* and other regulon members (27). Induction following DNA damage ranges between regulon members; *recA* and *lexA* are induced 10 and 2- to 5-fold, respectively (43–45). Similarly, DNA damage caused by UV-irradiation or mitomycin C treatment strongly induces *recA* transcript and protein accumulation in another cyanobacterium, *Anabaena variabilis* (46,47). Levels of both the *recA* transcript and its protein remain elevated until the damaging agents are removed and/or the DNA repaired, as observed for *E.coli* *recA* transcripts (27). In contrast, expression of the *Synechocystis* *recA* and *lexA* genes was not induced by UV-irradiation. Rather, our results show that *recA* and *lexA* levels decrease following UV-treatment, in agreement with other studies (39,48). These results indicate that the DNA damage induction of *recA* is variable among not only cyanobacteria but also prokaryotes in general. Our observation that *lexA* is not induced following UV-irradiation further suggests that *Synechocystis* LexA is not required for survival following DNA damage, and therefore potentially regulates expression of genes not associated with DNA repair. We therefore refer to this protein as being LexA-related.

The discrepancies in *lexA* regulation may also imply differences at the protein level, where LexA self-cleavage may not be required for derepression of gene expression. This appears to be the case, as *Synechocystis* LexA possesses modifications in two sites important for LexA function in *E.coli*; an altered cleavage site, and the absence of the nucleophilic serine of the Ser-Lys dyad. In *E.coli*, LexA self-cleavage and derepression of the SOS regulon requires a catalytic serine/lysine dyad and an Ala-Gly cleavage bond (31). In the absence of these residues, as indicated by mutational studies, LexA self-cleavage in *E.coli* is defective (31). These modifications to the *Synechocystis* protein have been previously noted as a potential explanation for the absence of a ‘cyanobacterial’ SOS box within the upstream regions of *Synechocystis* DNA repair genes (36), and further imply a unique cellular function for the *Synechocystis* LexA protein.

Novel roles for LexA have been implied in other bacteria, including *Mycobacterium tuberculosis* (49,50) and *Deinococcus radiodurans* (51), although the alternative function has not been identified. In *D.radiodurans*, RecA protein levels remain unchanged regardless of the *lexA* status (52), which is unexpected if LexA is required to regulate *recA* expression and is similar to the results reported here. DNA damage induction of repair genes in *M.tuberculosis* also occurs predominately via a LexA- and RecA-independent mechanism as shown by mitomycin C induction of DNA repair gene expression in *recA* mutants (49,50). Evidence for separation of *recA* expression from *lexA* regulation may also exist in higher plant chloroplasts, which possess a DNA damage-induced *recA* homologue (53), while *lexA* has not been reported to be encoded by plant genomes. This evidence suggests that conservation of the LexA/RecA regulation of the SOS response may be less widespread than previously anticipated, and furthermore, homologues of these proteins may fulfill different roles in their respective hosts.

crhR encodes an RNA helicase proposed to regulate RNA metabolism through its modification of RNA secondary structure (1,26). The redox-responsive regulation of *crhR* expression suggests that its cellular capacity to catalyze RNA secondary structure modifications is regulated by the redox status of the electron transport chain. The observed induction of *crhR* resulting from respiratory electron flow suggests that *crhR* may regulate the function of RNAs associated with photosynthesis (light harvesting and/or carbon metabolism) (1) or the cellular response to the predominating light/redox environment. The implication of *crhR* in carbon metabolism is consistent with both a proposed role for LexA in the regulation of carbon uptake and utilization genes (39) and observations demonstrating reduction in *lexA* transcript accumulation following a downshift in inorganic carbon availability (54). Further to our hypothesis suggesting LexA may ensure cells express the necessary gene products to respond to a dynamic light environment, one of the proposed functions for the bidirectional hydrogenase, a recently identified LexA-activated gene (37,38), is as an electron valve during photosynthesis (55). LexA regulation of *crhR* and the *hoxEFUYH* bidirectional hydrogenase may ensure continued maximal photosynthetic capabilities in response to changing cellular redox conditions.

Identification of a LexA-related protein as the regulator of *crhR* transcription provides unique insights into the

mechanism by which redox-regulated gene expression is controlled in photosynthetic cyanobacteria. The observations suggest a unique regulatory role for *Synechocystis* LexA in regulating gene expression in response to environmental cues other than DNA damage. These insights also imply the ubiquitous nature of the Lex/RecA DNA repair dogma is not conserved in *Synechocystis*, raising questions regarding the mechanisms by which DNA repair gene expression is regulated in this organism.

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