

Regulation of Cold Shock-Induced RNA Helicase Gene Expression in the Cyanobacterium *Anabaena* sp. Strain PCC 7120

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Expression of the *Anabaena* sp. strain PCC 7120 RNA helicase gene *crhC* is induced by cold shock. *crhC* transcripts are not detectable at 30°C but accumulate at 20°C, and levels remain elevated for the duration of the cold stress. Light-derived metabolic capability, and not light per se, is required for *crhC* transcript accumulation. Enhanced *crhC* mRNA stability contributes significantly to the accumulation of *crhC* transcripts, with the *crhC* half-life increasing sixfold at 20°C. The accumulation is reversible, with the cells responding more rapidly to temperature downshifts than to upshifts, as a result of the lack of active mRNA destabilization and the continuation of *crhC* transcription, at least transiently, after a temperature upshift. Translational inhibitors do not induce *crhC* expression to cold shock levels, indicating that inhibition of translation is only one of the signals required to activate the cold shock response in *Anabaena*. Limited amounts of protein synthesis are required for the cold shock-induced accumulation of *crhC* transcripts, as normal levels of accumulation occur in the presence of tetracycline but are abolished by chloramphenicol. Regulation of *crhC* expression may also extend to the translational level, as CrhC protein levels do not correlate completely with the pattern of mRNA transcript accumulation. Our experiments indicate that the regulation of *crhC* transcript accumulation is tightly controlled by both temperature and metabolic activity at the levels of transcription, mRNA stabilization, and translation.

Microorganisms respond to decreases in growth temperature via a process termed the cold shock response (7, 10, 24). In *Escherichia coli*, where cold shock has been most extensively studied, the cold shock response is initiated when the organism experiences a decrease in growth temperature of greater than 13°C (10). Physiologically, the response is divided into two phases, an initial lag in the growth or acclimation period followed by a resumption in growth. Sixteen gene products, termed cold shock proteins (CSPs), are specifically and transiently expressed during the acclimation phase in *E. coli* (24). CSPs include a diverse group of proteins involved in the transcription, translation, and function of mRNA, including CspA, polynucleotide phosphorylase (PNPase), RecA, initiation factor 2, CsdA, RbfA, NusA, histone-like nucleoid-structuring protein (H-NS), trigger factor, and GyrA (7, 10, 24).

Regulation of CSP expression in *E. coli* occurs at numerous levels, including transcription, mRNA stabilization, and translation, and has been best characterized for the most abundant CSP gene, *cspA*. At the transcriptional level, *cspA* is regulated positively by an AT-rich upstream element and negatively by a cold box (8, 15). *cspA* expression is regulated differentially at the posttranscriptional level by an unusually long 5' untranslated region (5' UTR) and a downstream box required for translation initiation (15). mRNA stabilization also plays a significant role in *cspA* transcript accumulation (2, 6, 10). Cold shock induction of *cspA* transcript accumulation does not require protein synthesis (4), as is indicated by the induction of CSP expression in *E. coli* cells exposed to translational inhibitors which interfere with ribosome function at 37°C (9, 25).

Although the mechanism regulating CSP expression is not

known, the rate-limiting step under cold shock conditions is the initiation of translation (10, 13). A cold shock ribosome adaptation model which allows this rate-limiting step to be overcome by the association of three CSPs, translation initiation factor 2, CsdA (RNA helix destabilization), and RbfA (ribosome binding factor A), with the ribosome, converting it into a cold-resistant translatable state, has been proposed (11, 24). In this scenario, *csdA*, which possesses RNA helix-destabilizing activity, is proposed to remove secondary structures in the highly structured 5' UTRs of *E. coli* CSP mRNAs, thereby facilitating translation initiation (12).

In cyanobacteria, a group of gram-negative photoautotrophic prokaryotes, only fatty acid desaturases (14, 18), ribosomal protein S21 (22), heat shock protein ClpB (17), and a family of RNA binding proteins (20, 21) are known to be expressed in response to cold shock. Investigation of the desaturase (14, 18) and RNA binding protein (20, 21) gene family members which respond to cold shock indicates that temperature-induced changes in mRNA stability and rates of transcription play major roles in the regulation of their expression.

We have recently reported that a cyanobacterial RNA helicase gene, *crhC*, is specifically induced by cold shock (3). Here we describe the regulation of *crhC* expression by temperature at the transcriptional, posttranscriptional, and translational levels and discuss the possible role(s) which CrhC may perform during cold shock adaptation in cyanobacteria.

MATERIALS AND METHODS

Growth and maintenance of organisms. *Anabaena* sp. strain PCC 7120 (referred to hereafter as *Anabaena*) was grown axenically in BG-11 medium at 30°C with a constant illumination of 150 microeinsteins/m²/s (3). Liquid cultures (300 ml) were aerated by a combination of shaking at 150 rpm on a rotary shaker and bubbling with air. Aliquots (50 ml) were aerated by shaking at 150 rpm on a rotary shaker and were treated as described in the figure legends. Cultures were cold induced for 1 h at 20°C unless otherwise stated. Antibiotics were obtained from Sigma, except for tetracycline (Boehringer Mannheim).

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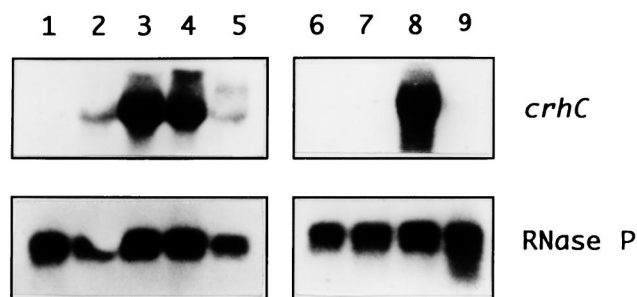


FIG. 1. Cold shock-induced increases in *crhC* transcript abundance is regulated by temperature and metabolic activity. Northern blots of total RNA (15 μ g) extracted from *Anabaena* exposed to different temperatures and/or light-dark conditions were hybridized with either the *crhC* or the RNase P gene. The autoradiograms are shown. Lane 1, 1 h at 30°C; lane 2, 1 h at 25°C; lane 3, 1 h at 20°C; lane 4, 1 h at 15°C; lane 5, 1 h at 10°C; lane 6, 3 h at 30°C in the dark, followed by 1 h at 20°C in the dark; lane 7, 4 h at 30°C in the dark; lane 8, 3 h at 30°C in the light, followed by 1 h at 20°C in the dark; lane 9, 4 h at 30°C in the light. The blots were probed with *crhC*, stripped, and reprobed with RNase P, as indicated.

RNA manipulations. Total RNA was isolated from *Anabaena* using glass bead lysis (20). Northern blots containing 15 μ g of total RNA per lane were generated and probed as previously described (3). Radioactive probes for detection of the *crhC* and the constitutively expressed *Anabaena* RNase P (as a control for RNA loading) gene transcripts by Northern blot analysis were produced as previously described (3). Autoradiograms were produced on X-ray film, while for quantification, *crhC* signals were corrected for RNA loading by comparison with those obtained with the constitutive RNase P probe using a PhosphorImager (Molecular Dynamics) and analyzed using ImageQuant 4.0 software.

Protein manipulations. Total protein was isolated from *Anabaena* by vortexing it in the presence of glass beads, in a buffer containing 50 mM Tris (pH 8), 100 mM EDTA, 0.5% Triton X-100, 0.5% Sarkosyl, and 0.4% sodium dodecyl sulfate, and quantified by the Bradford assay (Bio-Rad) using bovine serum albumin as the standard. Polypeptides (50 μ g) were separated on sodium dodecyl sulfate–10% polyacrylamide gels and transferred to nitrocellulose membranes (Bio-Rad) using a semidry apparatus (Tyler), and Western blot analysis was performed as described previously (1) with rabbit anti-CrhC antiserum (E. Yu and G. W. Owttrim, unpublished data) and goat anti-rabbit immunoglobulin G horseradish peroxidase conjugate (Cappel). Polypeptide size was determined by comparison with Kaleidoscope prestained molecular weight markers (Bio-Rad). The degree of inhibition of protein synthesis by antibiotics was determined by in vivo pulse-labeling of cells with a [³⁵S]methionine-cysteine mixture (Amersham ProMix) as described previously (1). Briefly, mid-log-phase *Anabaena* cells, grown at 30°C, were harvested and resuspended in an equal volume of sulfate-free BG-11. Cultures were incubated at the either 20 or 30°C for 5 min before the addition of antibiotics at the concentrations indicated in the figure legends, and incubation continued for 5 min. [³⁵S]Met-Cys (10 μ Ci/ml) was added, and incubation continued for 25 min (30 min total in the presence of antibiotics). Duplicate aliquots were treated, and ³⁵S incorporation into acid-insoluble material was quantitated as described previously (1). Control cultures, grown at either 20 or 30°C, were treated as described above except that the antibiotics were omitted.

RESULTS

***crhC* transcript accumulation is temperature and metabolism dependent.** We have previously shown that *crhC* transcripts are specifically detected after exposure of *Anabaena* cells to cold shock conditions (20°C) (3). In order to determine how growth temperature and light-dark cycles influence *crhC* transcript accumulation, Northern blot analysis was performed. The results indicate that *crhC* transcripts are not detectable at 30°C but that their abundance is differentially induced at temperatures below this level, with maximum abundance observed between 20 and 15°C (Fig. 1). *crhC* transcripts do not accumulate at temperatures higher than 25°C, including 30°C (Fig. 1, lane 1), 37°C (data not shown), and 43°C (3). Thus, *crhC* transcript abundance is finely regulated by minor changes in growth temperature.

Since *Anabaena* is an obligate photoautotroph, relying on photosynthetic light harvesting for growth and metabolism, we

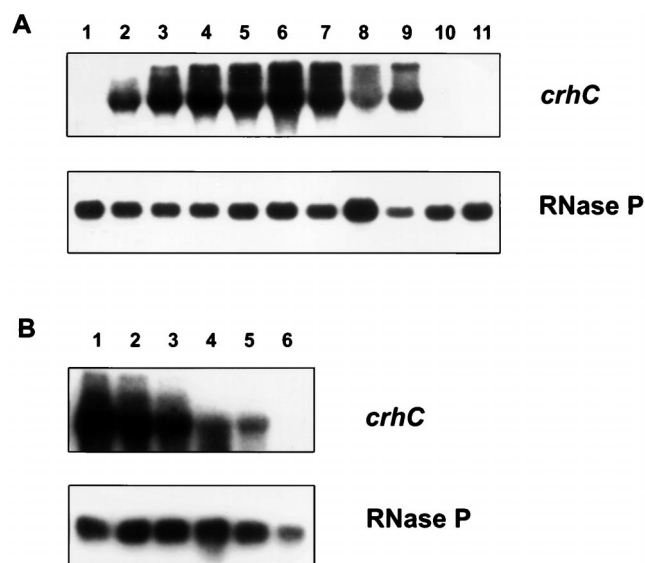


FIG. 2. Time course of cold-induced accumulation and warmth-induced decay of *crhC* transcripts. (A) Fifteen micrograms of total RNA extracted from each sample was subjected to Northern blot analysis using *crhC* and RNase P probes, as indicated. RNA was obtained from *Anabaena* cells grown at 30°C and then cold shocked at 20°C for the following lengths of time: 0 h (lane 1), 0.25 h (lane 2), 0.5 h (lane 3), 1 h (lane 4), 2 h (lane 5), 3 h (lane 6), 6 h (lane 7), 24 h (lane 8), and 48 h (lane 9). Lanes 10 and 11 contain RNAs from control cultures grown at 30°C for 24 and 48 h, respectively. (B) Northern blot analysis of total RNA (15 μ g) extracted from *Anabaena* exposed to 20°C for 1 h and then to 30°C for 0 h (lane 1), 0.25 h (lane 2), 0.5 h (lane 3), 1 h (lane 4), 2 h (lane 5), and 3 h (lane 6). The blot was probed with *crhC*, stripped, and reprobed with RNase P, as indicated.

asked if light was also required for the cold shock-induced accumulation of *crhC* transcripts. This appears to be the case, as *crhC* transcripts were not detected in cells which were incubated in the dark for 3 h before transfer to the cold (Fig. 1, lane 6), results identical to those obtained from cells incubated continuously at 30°C in the dark or light (Fig. 1, lane 7 or 9, respectively). Light is not required, however, for the accumulation of *crhC* transcripts if the *Anabaena* culture is transferred to the cold and dark simultaneously (Fig. 1, lane 8). Thus, it is not light per se but light-derived metabolic capability which is required for *crhC* transcript accumulation in response to cold shock.

***crhC* transcript and protein accumulation patterns differ.** In order to determine the pattern of cold-induced *crhC* transcript accumulation, a time course of *crhC* induction was determined. Northern blot analysis indicated that *crhC* transcripts are not detectable in cells grown at 30°C (Fig. 2A, lane 1) but that they accumulate within 15 min after transfer to 20°C (Fig. 2A, lane 2). *crhC* transcripts were expressed constitutively during growth at 20°C, reaching half-maximal levels 30 min after a temperature downshift. We have consistently observed that *crhC* transcript levels decrease fourfold 24 h after cold shock initiation (Fig. 2A, lane 8) but recover after 48 h (Fig. 2A, lane 9). *crhC* transcripts were not detectable in cells grown for 24 or 48 h at 30°C (Fig. 2A, lanes 10 and 11). Three RNA transcripts were detected by the *crhC* probe, a major transcript whose size corresponds to that expected for a full-length *crhC* transcript and two longer ones. Although all three transcripts were observed at all times during cold stress, the relative abundance of each transcript varied depending on the length of exposure to cold shock. Early in cold shock the abundance of the longest transcript was low relative to that of the middle transcript,

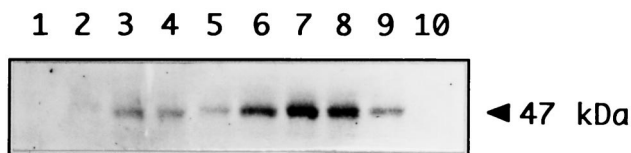


FIG. 3. CrhC protein expression mimics transcript accumulation. Total protein was extracted from *Anabaena* exposed to 20°C for various lengths of time. The Western blot, containing 50 µg of protein per lane and immunodecorated with rabbit anti-CrhC antiserum, is shown. Lane 1, 0 min; lane 2, 15 min; lane 3, 30 min; lane 4, 1 h; lane 5, 2 h; lane 6, 3 h; lane 7, 6 h; lane 8, 24 h; lane 9, 48 h; lane 10, 48 h at 30°C. The position of the 47-kDa CrhC protein is indicated by an arrow.

while after prolonged exposure the relative abundances of these transcripts reversed (Fig. 2A, compare lanes 3 and 9).

To determine if the cold-induced accumulation of *crhC* transcripts is reversible, a time course of *crhC* mRNA transcript decay after cold-induced cultures were transferred to 30°C was determined (Fig. 2B). *crhC* transcript levels declined after a temperature upshift and were no longer detectable within 3 h (Fig. 2B, lanes 2 to 6). *crhC* transcript abundance declined by 50% within 90 min of a temperature upshift. The results indicate that the cold-induced accumulation of *crhC* transcripts is fully reversible. Furthermore, *crhC* transcripts accumulate more quickly after a temperature downshift than they decay after a temperature upshift.

To determine if translational control plays a role in *crhC* expression, the relationship between the abundance of *crhC* mRNA transcripts and CrhC protein levels was determined. Western blot analysis of a time course of cold-induced CrhC protein production (Fig. 3) shows that the induction of CrhC polypeptide corresponds to transcript accumulation early in cold shock but not during prolonged exposure to low temperature. The *crhC* gene product was not detected in cells grown continuously at 30°C (Fig. 3, lanes 1 and 10) but was detectable within 15 min after transfer to 20°C (Fig. 3, lane 2) and remained elevated for the duration of the cold treatment (Fig. 3, lanes 2 to 9). While transcript levels decreased after 24 h of cold shock and recovered by 48 h, protein levels were reversed, being maximal after 24 h, and decreased at 48 h. It should be noted that growth at 20°C for longer than 48 h resulted in the cells becoming achlorotic, as was also seen for *Anabaena variabilis* strain M3 (21).

***crhC* transcripts are stabilized in the cold.** The *crhC* transcript half-life was determined to ascertain the contribution that temperature-induced changes in mRNA stability make to *crhC* transcript accumulation. The transcriptional inhibitor rifampin was added to cold-induced cultures before continued incubation at 20°C or transfer to 30°C, and *crhC* transcript levels were determined at the indicated times thereafter (Fig. 4). Linear regression analysis of changes in *crhC* transcript levels over time indicated that the *crhC* transcript has a sixfold longer half-life at 20°C (67 min) than at 30°C (11 min). mRNA

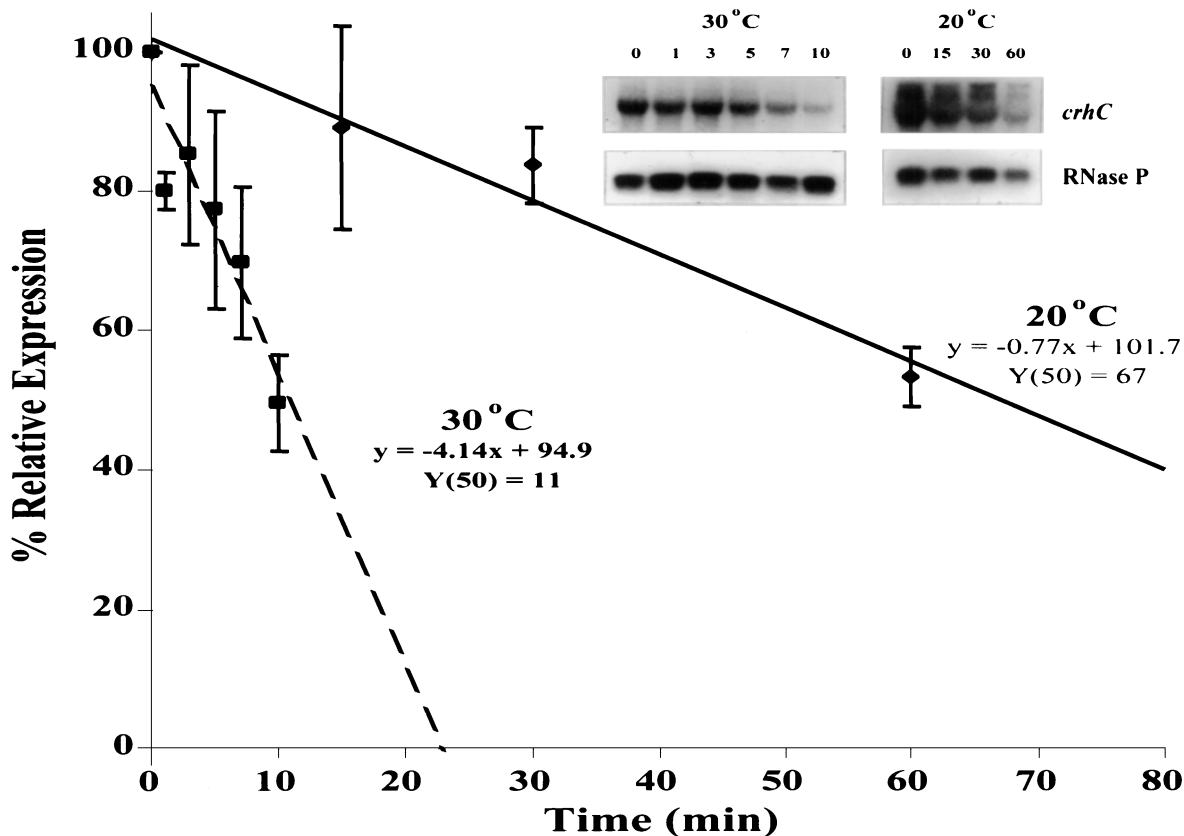


FIG. 4. *crhC* transcripts are stabilized at 20°C. Total RNA (15 µg) was extracted from *Anabaena* exposed to 20°C for 1 h, followed by the addition of rifampin (400 µg/ml) and either continued exposure at 20°C or transferred to 30°C for the indicated times. *crhC* transcript levels, determined by Northern blot analysis, were quantitated to determine their half-lives at the respective temperatures. Average values from triplicate, independent repetitions of the experiments were subjected to linear regression analysis and are plotted (squares and hatched line, 30°C; diamonds and solid line, 20°C). Shown in the inset are representative autoradiograms of Northern blots used to generate the data points.

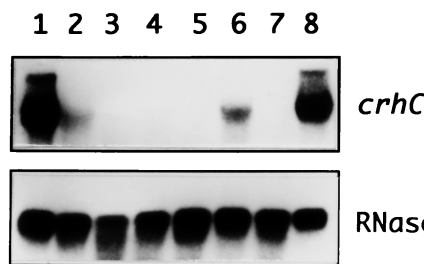


FIG. 5. Cold shock-mimicking translational inhibitors partially induce *crhC* transcript accumulation at 30°C. The autoradiogram of a Northern blot of total RNA (15 µg) extracted from *Anabaena* exposed to various cold shock-mimicking translational inhibitors for 30 min at 30°C is shown. Lanes 1 and 8, 30 min at 20°C, no inhibitor; lane 2, chloramphenicol (10 µg/ml); lane 3, erythromycin (500 µg/ml); lane 4, fusidic acid (0.5 µg/ml); lane 5, spiramycin (800 µg/ml); lane 6, tetracycline (10 µg/ml); lane 7, ethanol (0.8%) as a control without antibiotics. The blots were probed with *crhC*, stripped, and reprobed with RNase P, as indicated.

stability, therefore, plays a significant role in the regulation of *crhC* transcript accumulation.

Temperature-induced alterations in the rate of *crhC* transcription may also play a role in the observed effects on *crhC* transcript accumulation. To test this possibility, we used PhosphorImager quantitation to compare the rates of *crhC* transcript degradation at 30°C, after a temperature upshift, in the presence and absence of rifampin. *crhC* transcript levels decreased to half-maximal levels within 90 min in the absence of rifampin, while similar levels were reached within 11 min in the presence of rifampin. These results suggest that *crhC* transcription continues after a temperature upshift. Continued transcription of *crhC* after a temperature upshift may occur only temporarily, as transcript accumulation was no longer detectable within 3 h after a temperature upshift (Fig. 2B, lane 6).

Protein synthesis is required for *crhC* transcript accumulation. Temperature-induced changes in *crhC* mRNA stability and transcription may require protein synthesis. Since inhibitors of protein synthesis mimic the cold shock response in *E. coli*, we first determined *crhC* transcript levels in *Anabaena* cells grown at 30°C after exposure to five translational inhibitors. All of the inhibitors induced *crhC* transcript levels marginally at 30°C, although some were at a level which was too low to be reproduced photographically (Fig. 5, lanes 2 to 6). Ethanol, which was used to dissolve the inhibitors, did not induce expression (Fig. 5, lane 7). Although chloramphenicol and tetracycline, at 10 µg/ml, induced *crhC* transcript accumulation most efficiently of the five translational inhibitors tested, the level of accumulation was only 15 to 20% of that observed under cold shock conditions (Fig. 5, compare lanes 1 and 8 with 2 and 6). At 30°C, the effects of the inhibitors appeared to be concentration dependent, as comparable results were obtained using tetracycline at 10 µg/ml (Fig. 5, lane 6) and 40 µg/ml (data not shown), while chloramphenicol did not induce expression at 40 µg/ml (data not shown). Thus, inhibition of translation only partially mimics the cold shock response and protein synthesis may be required for the partial induction of *crhC* transcript accumulation by translational inhibitors at 30°C.

To ensure that these observations were a result of translational inhibition, we determined the level of protein synthesis in *Anabaena* cells grown at 20 and 30°C in the presence of chloramphenicol and tetracycline at 10 and 40 µg/ml. At 20°C, chloramphenicol inhibited protein synthesis by 69% at 10 µg/ml while it inhibited synthesis by 91% at a concentration of 40 µg/ml. Tetracycline inhibited protein synthesis by 94 and

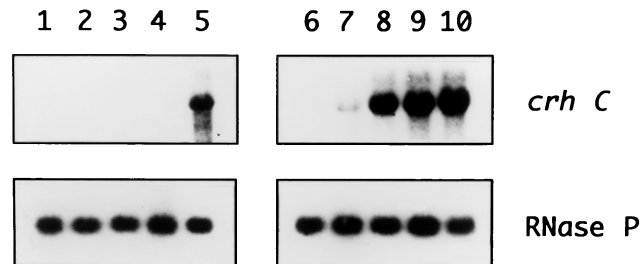


FIG. 6. Translational inhibitors differentially affect *crhC* induction at 20°C. The autoradiograms of Northern blots of total RNA (15 µg) extracted from *Anabaena* exposed to either chloramphenicol (40 µg/ml, lanes 1 to 4) or tetracycline (10 µg/ml, lanes 6 to 9) at 20°C for the indicated lengths of time are shown. Lanes 1 and 6, 0 min; lanes 2 and 7, 10 min; lanes 3 and 8, 20 min; lanes 4 and 9, 30 min; lanes 5 and 10, 30 min at 20°C without inhibitor. The blots were probed with *crhC*, stripped, and reprobed with RNase P, as indicated.

97% at final concentrations of 10 and 40 µg/ml, respectively. Temperature did not affect the level of inhibition, as comparable results were observed at 30°C.

We then asked whether protein synthesis is required for *crhC* transcript accumulation under cold shock conditions. Northern blot analysis was performed on RNAs isolated from cultures exposed to either chloramphenicol or tetracycline at 20°C (Fig. 6). Inhibition of protein synthesis by chloramphenicol at 40 µg/ml suppressed the cold shock-induced accumulation of *crhC* transcripts (Fig. 6, lanes 1 to 4). As a control, the partial inhibition of protein synthesis by chloramphenicol at 10 µg/ml did not suppress *crhC* transcript accumulation (data not shown). Conversely, cold-induced *crhC* transcript accumulation was not affected by tetracycline at 10 µg/ml (Fig. 6, lanes 6 to 9) and was only marginally reduced by 40 µg/ml (data not shown), inhibitor concentrations which essentially abolished protein synthesis. Furthermore, both the time dependency and the degrees of cold-induced *crhC* transcript accumulation were found to be comparable in the presence and absence of tetracycline. Thus, a limited amount of protein synthesis is required for cold shock induction of *crhC* transcript accumulation in *Anabaena*.

DISCUSSION

Free-living prokaryotic organisms must have the capacity to respond rapidly to fluctuations in growth temperature. These responses are regulated at the molecular level and have been characterized for heat shock but not nearly as well for cold shock (24). The majority of cold shock-induced gene products affect the translational machinery or membrane fluidity (24). Recently, we have shown that *crhC*, which encodes a cyanobacterial RNA helicase, is expressed under cold shock conditions (3). In this report we investigate the regulation of cold-induced *crhC* expression at the transcriptional, posttranscriptional, and translational levels.

At the metabolic level, the cold-induced expression of *crhC* does not require light directly, as transcript accumulation occurs if the cells are transferred to cold and dark conditions simultaneously. Similar results have been reported for the cold-induced expression of the *A. variabilis* M3 RNA binding protein gene *rbpA1* (21). *crhC* expression, however, does require light-derived metabolic capability, since *crhC* transcript accumulation does not occur if the temperature downshift occurs 3 h after initiation of the dark treatment. During this dark period, cyanobacterial metabolism is downregulated to a sig-

nificant degree, corresponding to the time when photosynthetically derived glucose levels are depleted (16).

In the presence of adequate metabolic activity, multiple *crhC* transcripts accumulate after a temperature downshift and remain elevated for the duration of the cold stress. The relative abundance of the individual *crhC* transcripts is altered by the length of exposure to cold stress. This observation suggests that there may be two phases of *crhC* cold shock regulation in *Anabaena*, a rapid initial response within 3 h and a long-term response after 24 h. The presence of three hybridizing transcripts may also indicate that *crhC* is expressed as part of a cold shock operon. Multiple transcripts, whose origins have not been determined, have also been observed for both the *Synechocystis* sp. strain PCC 6803 (14) and the *Synechococcus* sp. strain PCC 7002 (18) *desC* desaturase genes, whose constitutive expression is enhanced by cold shock. Although *crhC* is constitutively expressed for the duration of the cold stress, transcript levels decrease fourfold 24 h after initiation of the cold treatment but recover after 48 h. This observation may indicate the additional involvement of a secondary stress or circadian rhythm in the regulation of *crhC* transcript accumulation during prolonged exposure to low temperature. The pattern of *crhC* transcript induction after a temperature downshift is similar to that observed for the *A. variabilis* M3 cold-regulated RNA binding proteins *rbpA1* and *rbpA2* over a 25-h time course (20).

The half-rise time of *crhC* transcript accumulation is similar to that observed for the cold-induced accumulation of *desA* transcripts in *Synechocystis* sp. strain PCC 6803 (14). Furthermore, the cold-induced accumulation of *crhC* transcripts is completely reversible, with a time frame of decay which is slower than that required for *crhC* transcript accumulation to reach half-maximal levels after a temperature downshift. This reduction in the rate of *crhC* transcript decay after a temperature upshift may result from the continuation of transcription and/or lack of active *crhC* mRNA destabilization at 30°C (see below).

Changes in mRNA stability are important for the temperature regulation of *crhC* expression, as the *crhC* transcript half-life is enhanced sixfold by cold stress. This degree of *crhC* mRNA stabilization is similar to that observed for other cold-induced cyanobacterial genes, whose stability increases between 3.5- and 15-fold upon a temperature downshift (14, 18, 21). Temperature-induced changes in mRNA stability cannot be accounted for solely by thermodynamic considerations, as the mRNA half-lives of other cold-enhanced gene family members are differentially affected by temperature, for example *desC*, whose half-life is not affected by growth temperature (14, 18). In addition, cyanobacterial RNase activity does not appear to be affected by alterations in environmental conditions, as it has recently been shown that varied light regimens do not affect RNase activity in *Synechococcus* sp. strain PCC 7002 (19).

The regulation of temperature-induced changes in cold shock gene mRNA stability may involve either stabilization at 20°C or destabilization at 30°C. RNA stabilization is known to play a major role in the regulation of mRNA levels for a number of CSP genes, although the proposed mechanisms responsible for this control vary (2, 6, 7, 14, 18, 21, 24). The decrease in *crhC* mRNA stability when transcription is inhibited by rifampin is consistent with *crhC* mRNA stabilization at 20°C and rules out the involvement of an mRNA-destabilizing factor, as has been proposed to regulate *desB* levels in *Synechococcus* sp. strain PCC 7002 at 38°C (18).

Temperature-induced changes in *crhC* transcription may also be involved in *crhC* transcript accumulation. *crhC* tran-

scription occurs at least transiently after a temperature upshift, as *crhC* transcripts are more stable in the absence of rifampin than in its presence. Regulation of cold-induced transcript accumulation by changes in transcriptional activity has been proposed for other prokaryotic genes, including *cspA* from *E. coli* (6), *rbpA1* from *A. variabilis* M3 (21), and *desA* and *desB* from *Synechococcus* sp. strain PCC 7002 (18) and *Synechocystis* sp. strain PCC 6803 (14).

Translational inhibitors only marginally mimicked the cold-induced accumulation of *crhC* transcripts at 30°C. The limited induction may be concentration dependent, as chloramphenicol at concentrations which abolished translation did not elicit *crhC* transcript accumulation. Protein synthesis, therefore, may be required for a limited amount of *crhC* transcript accumulation at 30°C. These results suggest that the inhibition of protein synthesis is only one component of the signal required for the induction of *crhC* transcript accumulation by cold stress in *Anabaena*. In contrast, translational inhibitors and cold shock induce CSP expression by similar mechanisms in *E. coli*, as a low concentration of inhibitor mimicked CSP expression to cold shock levels at 37°C (24) while high concentrations of chloramphenicol only marginally induced expression (8). Thus, while similar mechanisms exist for CSP expression induced by cold shock and antibiotic inhibition of translation in *E. coli* (8), the two mechanisms appear to operate via different mechanisms in *Anabaena*.

A limited amount of protein synthesis is required for maximal *crhC* transcript accumulation at 20°C, as increased *crhC* transcript abundance is observed in the presence of tetracycline but not chloramphenicol at levels which reduce translation by greater than 90%. Cold shock-induced accumulation of *crhC* transcripts in the presence of chloramphenicol at levels which only partially inhibit translation is also consistent with the requirement for protein synthesis. The requirements for protein synthesis for the cold induction of transcript accumulation differ between the CSP genes. Protein synthesis is required for the accumulation of *desA* and *desB* transcripts in *Synechococcus* sp. strain PCC 7002 (18) but not for *desC* in *Synechococcus* sp. strain PCC 7002 (18), *rbpA1* in *A. variabilis* strain M3 (21), or *cspA* in *E. coli* (4).

The antibiotic effects are not a result of the level of protein inhibition, as both antibiotics inhibit translation to comparable levels. They may, however, be a reflection of the underlying mechanism regulating *crhC* transcript accumulation. Tetracycline and chloramphenicol differentially affect translation; tetracycline inhibits initiation and not elongation, while chloramphenicol blocks only elongation (23). Cold-induced *crhC* transcript accumulation in the presence of tetracycline and not chloramphenicol therefore suggests that a factor whose expression is required for *crhC* transcript accumulation is transcribed and translated at 30°C but that its gene products are unstable at this temperature. Potential candidates for this factor include a derepressor of transcription, a possibility that is consistent with our preliminary observation of a protein associated with the *crhC* promoter at 30°C but not 20°C (R. Blush and G. W. Owttrim, unpublished data). Furthermore, the *crhC* promoter contains sequences (3) known to regulate transcription of cold shock genes in *E. coli* (5, 15). A model involving derepression of transcription by protein modification of a repressor has been proposed to regulate the cold-induced expression of *rbpA1* in *A. variabilis* strain M3 (21).

Posttranscriptional regulation of *crhC* expression may also occur at the level of translation, as CrhC protein accumulation does not mimic *crhC* transcript abundance during prolonged exposure to low temperature. It is also possible that CrhC RNA helicase activity is required for its own translation in that

it may unwind the secondary structure in its highly structured 5' UTR (3), as has been proposed for CsdA in the *E. coli* cold shock ribosome adaptation model (11, 24).

The results presented here indicate that the cold-induced accumulation of *crhC* transcripts is tightly regulated by fine changes in growth temperature at the levels of metabolic activity, transcription, mRNA stabilization, and translation. Although the pattern of *crhC* transcript accumulation is similar to temperature-induced alterations in the levels of abundance of transcripts of other cold shock genes, the mechanisms by which this is accomplished appear to differ. We are currently investigating the factors regulating *crhC* expression during acclimation to cold stress in *Anabaena* and the role performed by CrhC in this process.

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