Transcript analysis of the \textit{pcbABC} genes encoding the antenna apoproteins in the photosynthetic prokaryote, \textit{Prochlorothrix hollandica}

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Abstract

The tightly linked \textit{pcbABC} genes encode the chlorophyll a/b-binding apoproteins in the oxygenic photosynthetic prokaryote \textit{Prochlorothrix hollandica}. Northern blotting experiments employing gene-specific DNA probes have identified a complex pattern of transcription from the \textit{pcb} region. A large 4.4-kb transcript detected in cultures maintained in high light, low light and in darkness results from the cotranscription of all three genes, whereas \textit{pcbAB}, \textit{pcbBC} and individual \textit{pcbA}, B, and C mRNAs are similarly detected in all light regimes. The half lives of the RNAs vary from 15 min for the \textit{pcbABC} transcript, to over 60 min for the \textit{pcbA} and \textit{pcbC} mRNAs. The lack of identifiable promoter sequences other than the region upstream from \textit{pcbA}, plus the enhanced stability of the individual single gene transcripts, suggest that the smaller RNA species arise from processing of larger transcripts. Transcription and mRNA turnover occurs largely independent of light intensity, in contrast to what is seen in most other phototrophs, in which light influences the accumulation of antenna apoprotein gene mRNAs. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Photosynthetic antenna; Cyanobacteria; Transcript stability; Promoter; \textit{Prochlorothrix}

1. Introduction

The photosynthetic prokaryote, \textit{Prochlorothrix hollandica} is a divergent member of the cyanobacteria that has a chlorophyll \textit{a/b}-containing photosynthetic antenna instead of a phycobilisome light-harvesting system [1]. Previous work has identified a small family of 33–35-kDa polypeptides that comprise the apoproteins binding the antenna chlorophylls [2,3]. Such an antenna system is structurally distinct from the LHCII/LHCI chlorophyll \textit{a/b} antennae of higher plant chloroplasts, as the apoproteins are structurally homologous to the CP43/IsiA family of pigment-proteins [4]. In \textit{Prochlorothrix} sp., three tightly linked homologous genes, \textit{pcbABC}, encode the antenna apoproteins [4,5]. The PcbA and PcbB proteins are highly homologous, but the PcbC protein is a divergent protein more similar to the IsiA protein accumulating in iron-stressed cyanobacteria [5]. What role each of these polypeptides plays in light-harvesting is a current area of investigation.

Whereas there are three different genes in the \textit{pcb} gene family, only one protein (the \textit{pcbA} gene prod-
uct) is the dominant component of *P. hollandica* chlorophyll--protein complexes resolved on non-denaturing green gels (Nikolaitchik, Burkhart and Bullerjahn, in preparation). The accumulation and function of the PcbB and PcbC gene products has yet to be well established, due to the lack of available protein-specific antibodies. However, the mRNA species transcribed from the corresponding genes can be easily monitored, giving valuable information about mRNA production and stability during different light intensities and growth regimes. In this paper, we document that transcription of the *pcbABC* gene cluster yields a complex pattern of stable transcripts.

2. Materials and methods

2.1. Strain and growth conditions

*P. hollandica* ACC15-2 was grown in BG-11 medium [6] with constant illumination (medium light, 60 µmol quanta m\(^{-2}\) s\(^{-1}\)) and gentle aeration at 25°C. Under these conditions cells grew with a 20 h doubling time. For several experiments, cell cultures were transferred to the following illumination intensities: high light (HL), 235 W mol quanta m\(^{-2}\)s\(^{-1}\); low light (LL), 35 W mol quanta m\(^{-2}\)s\(^{-1}\); and in complete darkness.

2.2. DNA and RNA gel electrophoresis

DNA and RNA were extracted with the TRIZOL reagent (Gibco BRL) [7]. RNA molecules were separated on denaturing 1.2% agarose gels containing formaldehyde as described in Sambrook et al. [8]. DNA and RNA agarose gels were blotted under vacuum (Bio-Rad) according to the manufacturer’s instructions.

2.3. PCR primers for the *pcbA*, *B* and *C* probes

Since the *pcb* genes encoding antenna apoproteins in *P. hollandica* exhibit a high degree of homology to one another, the PCR-derived gene-specific probes were designed to hybridize to regions having the most diversity in nucleotide sequence. All nucleotide numbering is taken from the annotated nucleotide sequence file X97043 of the cloned *pcbABC* fragment [4,5].

The PCR primers for the *pcbA* probe were: 5’-ATGGCAACAACACTGCTACGCC-3’ hybridizing to nucleotides 343–362 and 5’-GGGCTGCTAACC-TAAGGTG-3’ hybridizing to nucleotides 780–799. PCR primers amplifying a *pcbB*-specific probe were: 5’-CAACACATACGGAAGTGGATTGT-3’ hybridizing to nucleotides 1594–1616 and 5’-CGCTACCCTCACAATGCTTTC-3’ hybridizing to nucleotides 1936–1957. The PCR primers for the *pcbC* probe were the following: 5’-GCCTCGAT-GAACAATGG-3’ hybridizing to nucleotides 2840–2856 and 5’-GGATCCCCAACAATAGG-3’ hybridizing to nucleotides 3255–3271. For each PCR, 50 ng of *P. hollandica* template DNA was used, along with 100 pmol of each primer. The annealing temperature was 63°C and extension time was 1 min.

PCR products were cloned into pGEN-T Eazy vector (Promega). The identity of *pcbABC* PCR products was determined by dideoxy DNA sequencing [8] and analysis of DNA sequences employed standard computer programs [9]. Nucleic acid secondary structure predictions were carried out using services available on the Internet at www.genebee.msu.su.

2.4. RNA turnover studies

Rifampicin was added to a medium light mid log phase *P. hollandica* culture to a final concentration of 150 µg/ml. Cells for zero time point samples were taken prior to the addition of the inhibitor. The culture was split into separate flasks and shifted to HL, LL or dark conditions. Sample aliquots of 50 ml were taken every 10 min for 1 h and centrifuged at 5000 x g for 15 min. RNA isolation and Northern analysis were conducted as described above.

2.5. Primer extension

The following primers were used for the primer extension studies [10]: (a) PCB1RT (5’-CAATGCG-TATTGCGGATGTGGGCGG-3’) hybridizing to nucleotides 421–447 of the cloned *pcbABC* region; (b) PCB2RT (5’-GCAAGCTAGACCGTGATC-3’) hybridizing to nucleotides 1651–1676 of the cloned *pcbABC* region; and (c) PCB3RT
3. Results

3.1. Northern hybridization to pcbA, B and C probes

Three identical nitrocellulose strips from the same gel were probed with pcbA, B and C probes (Fig. 1). Northern blot hybridization to the pcbA probe yielded three bands of approximately 4.4, 2.5 and 1.3 kb, with the 1.3-kb signal being the most intense (Fig. 1, lanes 1 and 2). The pcbB probe hybridized to two major RNA species corresponding to 4.4 and 2.5 kb in size with the 2.5-kb species being dominant (Fig. 1, lanes 3 and 4). Also, the pcbB probe sometimes also yielded a faint hybridization signal corresponding a 1.4-kb RNA species (Fig. 1, lanes 3 and 4). The pcbC probe gave faint hybridization signals arising from 4.4- and 2.2-kb mRNAs and a stronger signal at about 1.1–1.3 kb (Fig. 1, lanes 5 and 6).

Lastly, the pattern of transcription was independent of light intensity, as mRNAs from cells adapting to HL and LL conditions yielded similar hybridization patterns (Fig. 1; compare lanes 1, 3 and 5 to lanes 2, 4 and 6). Lastly, a band of approximately 2.2 kb was infrequently detected on Northern blots (Fig. 1A, lanes 3–6). It is possibly a pcbBC transcript present at low levels. The smallest mRNA species on Northern blots (1.3-, 1.4- and 1.2-kb mRNAs on the pcbA, B and C blots, respectively) most likely come from single gene transcripts.

3.2. mRNA turnover experiments

The addition of rifampicin to HL, LL and dark-incubated cultures was performed to assess the overall stability of the pcb transcripts. Fig. 2A,B shows the stability of the mRNAs hybridizing to the pcbA and C probes, respectively. Of particular note is the appearance of increased levels of the smallest pcbC transcript following transcription inhibition by rifampicin (Fig. 2B), strongly suggesting that the 1.4 and ca. 1.2-kb transcripts may have arisen by post-transcriptional processing of larger mRNA species. Overall, the smallest transcripts yielded the longest

(5’-GCTGACCCGACAGTCTACGAGGCG-3’)
hybridizing to nucleotides 2872–2896 of the cloned pcbABC region.

Reverse transcriptase products were electrophoresed in parallel with a sequencing reaction prepared with the same primer (PCB1RT) used for the pcbA primer extension experiment. Sizes of the primer extension products were determined by comparison to the sequencing ladder. The pAM1464 clone containing the pcbA, B and C genes (a generous gift from Dr. S. S. Golden) was used as DNA template.
half lives (~40 min), whereas the larger transcripts had half lives in the range of 15–20 min. Quantitation of Northern blots yielded the data of Table 1, summarizing the stabilities of pcb transcripts under HL, LL and dark conditions. Overall, transcript stability was not markedly affected by growth irradiance. Additionally, transcription is not dependent on photosynthetic activity, as de novo transcription was seen after 6–12 h in the dark, and DCMU treatment of LL cells still yields detectable transcripts after 2 h (data not shown). The life-time of pcb transcripts in the dark is also similar to that seen in light-grown cultures (Table 1).

3.3. Primer extension studies

All three primer extension reactions were run on a sequencing polyacrylamide gel next to the sequence ladder created with the PCB1RT pcbA-specific primer used as a size standard (Fig. 3). The actual 5’ ends of the pcbB and C transcripts were deduced according to sizes of the primer extension products and known pcbB and C gene sequences. A single putative transcription start site was determined at G230, 113 nucleotides upstream from the start of the pcbA translated region (Fig. 4A). Also, the 5’ end of a pcbB-containing transcript was found at C1515, 76 nucleotides upstream from the start of the pcbB translation start site (Fig. 4B). The 5’ end of a pcbC transcript was mapped to G2723, 56 nucleotides upstream from the start of the pcbC translation initiation codon (Fig. 4C).

These results give a calculated length of a single pcbA transcript of 1.3 kb which is in good agreement with Northern hybridization data. The size of single pcbB transcript was estimated at 1.1 kb, assuming the 3’ end of the pcbB transcript maps prior to the 5’ end of the pcbC mRNA. However, from Northern blots, the calculated size of the minor pcbB transcript is 1.4 kb. The size of the pcbC transcript is in agree-

Table 1

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<th>Probe</th>
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<td>1.1 kb</td>
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ment with the length of the pcbC coding sequence (c. 1.2 kb).

3.4. Sequence analysis within the pcbABC genomic region

Finding mRNA species of different length containing all three, two and one gene transcripts may indicate the presence of internal promoters, terminators or RNase processing sites within the pcbABC region.

The existence of specific promoter consensus sequences for cyanobacterial promoters is still under debate. The most recent analysis of sequence upstream from transcription start site for many cyanobacterial genes have not revealed any particular consensus sequences for a −35-like box ([11, 12], Bryant, D.A., personal communication). The consensus sequence TA(N3)T was proposed to be a functional cyanobacterial −10-like promoter [11, 12]. Examining the pcbABC operon, sequence elements which resemble this consensus, and hence σ30-dependent pro-

Fig. 4. Non-translated regions upstream from pcbA (A), pcbB (B) and pcbC (C), indicating the 5’ ends of the pcbA, B and C transcripts (asterisks), a putative promoter site upstream from pcbA (underlined) and sequences yielding potential stable secondary structures (arrows). Putative ribosome binding sites are also indicated (bold). The methionine initiation codons are italicized. Analysis of the sequence yields no identifiable internal promoter upstream from pcbB or pcbC.
motors in *Escherichia coli*, were found upstream from only the *pcbA* gene (Fig. 4A).

### 3.5. Potential mRNA secondary structures within the *pcbABC* genomic region

RNA secondary structure calculations indicated the presence of a 8-bp stem-loop structure downstream from the end of *pcbB* translated region. It exhibits the features of an intrinsic rho-independent terminator: a GC stretch and run of U residues at the end of the stem. However, if this hairpin is assumed to be a transcription terminator for *pcbAB* and/or *pcbB* mRNAs, then the calculated length of these mRNAs would be about 2.4 and 1.2 kb, respectively, which is about 200 bp shorter than 2.5- and 1.4-kb bands detected on the Northern blots.

Since the 5′ ends of the mRNAs were mapped by primer extension, one can assume that the 3′ ends of *pcbB*-containing mRNAs are extended downstream beyond the putative hairpin to the start of the *pcbC* coding region. Further analysis of the RNA secondary structure in the beginning of the *pcbC* coding sequence did not yield any sequence that could serve as an intrinsic terminator (Fig. 4).

Secondary structures of RNA can serve not only as terminators, but also as regulators of RNA processing events. Several riboendonucleases studied to date cleave RNA at the single strand at the base of the stem-loop, or at the mismatches within hairpin [13,14]. Thus, hairpins at the 3′ and at the 5′ ends of transcripts could act to protect mRNA from exonuclease attack increasing the stability of the mRNA [15]. Analysis of the intergenic region between *pcbA* and *B* revealed extended palindromic repeats between positions 1468 and 1519 that do not exhibit features of an intrinsic terminator, yet the 5′ end of the *pcbB*-containing mRNAs is contained within this potential secondary structure (Fig. 4B). However, the overall free energy of the structure is quite low (−21.5 kcal/mol). A consistent hairpin structure was also found at the 5′ end of the *pcbA* transcript (nucleotides 248–305) (Fig. 4A). Thus, both this hairpin and the structure at nucleotides 1468–1519 (Fig. 4B) could be involved in processing of the multigene transcript leading to smaller, more stable fragments [14–16].

### 4. Discussion

The results presented in this paper show that specific probes for *pcbA*, *B* and *C* genes yield multiple hybridization signals on Northern blots, suggesting a complex pattern of expression of the genes encoding antenna apoproteins in *Prochlorothrix hollandica*. Fig. 5 summarizes the likely origin of the transcripts detected by Northern hybridization. The 4.4-kb mRNA is longer than the known region containing the *pcb* coding sequence [4,5], suggesting that it arises from cotranscription of all three genes (Fig. 5). This is supported by the fact that the 4.4-kb mRNA hybridizes to all *pcb A*, *B* and *C* probes. The 2.5 kb signal appears on both *pcbA* and *pcbB*, but not on the blot probed with the *pcbC* probe (Fig. 1A,B). Calculated from the known DNA sequence, the length of translated *pcbAB* region comes to about 2.4 kb, thus suggesting that 2.5-kb mRNA comes from cotranscription of *pcbA* and *B* (Figs. 1 and 5).

The mechanism(s) regulating the differential accumulation of antenna gene transcripts is still unclear. However, our data suggest that the multiple transcripts arise via alternative internal terminators.
(as in the case of pcbB-containing transcripts) or, more likely, through processing of the multicis-tronic transcripts and stabilization of smaller mRNAs.

Even though the PcbA gene product is found to be the dominant antenna polypeptide (Nikolaitchik, Burkhart and Bullerjahn, in preparation), the presence of the pcbB and pcbC-containing transcripts were also detected during these studies. The size and appearance of the hybridization signals indicate that a multigenic transcript covering all three genes exists in cells along with two gene (pcbAB and pcbBC) and single gene (pcbA, pcbB and pcbC) transcripts. Additionally, the smaller mRNAs exhibited a longer half-life compared to the larger mRNAs that likely give rise to the smaller transcripts by processing events. Overall, the 1.4-kb pcbA and ca. 1.2-kb pcbC mRNA species showed a life-time comparatively long for a prokaryotic mRNA. The fact that such lifetimes were similar in high light, low light and dark-adapted cells also suggests that mRNA turnover does not play a major role in regulating pcb expression.

Overall, the expression pattern of the pcb genes does not appreciably change under different illumination conditions; this differs from other oxygenic phototrophs, in which light-dependent control and enhancement of transcription has been well documented (reviews [17,18]). The future availability of PcbA, B and C-specific antibodies will help resolve whether the polypeptide composition of the antenna changes during light/shade adaptation. Nonetheless, the continued abundance of pcbABC transcripts (this paper) and antenna proteins [19] in all light conditions suggests that regulation of antenna function in Prochlorothrix involves the rearrangement of long-lived existing antenna complexes and modulation of PSI/PSII levels, as had been proposed earlier from physiological studies [20]. Such a lack of control by light may explain in part the observed sensitivity of Prochlorothrix and other prochlorophytes to high irritants [1].

Acknowledgments

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References


