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REGULAR PAPER

The PucC protein of *Rhodobacter capsulatus* mitigates an inhibitory effect of light-harvesting 2 α and β proteins on light-harvesting complex 1

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Abstract *Rhodobacter capsulatus* contains *lhaA* and *pucC* genes that have been implicated in light-harvesting complex 1 and 2 (LH1 and LH2) assembly. The proteins encoded by these genes, and homologues in other photosynthetic organisms, have been classified as the bacteriochlorophyll delivery (BCD) family of the major facilitator superfamily. A new BCD family phylogenetic tree reveals that several PucC, LhaA and Orf428-related sequences each form separate clusters, while plant and cyanobacterial homologues cluster more distantly. The PucC protein is encoded in the *pucBACDE* superoperon which also codes for LH2 α (PucA) and β (PucB) proteins. PucC was previously shown to be necessary for formation of LH2. This article gives evidence indicating that PucC has a shepherding activity that keeps the homologous α and β proteins of LH1 and LH2 apart, allowing LH1 to assemble properly. This shepherding function was indicated by a 62% reduction in LH1 levels in ΔLHII strains carrying plasmids encoding *pucBA* along with a C-terminally truncated *pucC* gene. More severe reductions in LH1 were seen when the truncated *pucC* gene was co-expressed in the presence of C-terminal PucC::PhoA fusion proteins. It appears that interaction between truncated PucC::PhoA fusion proteins and the truncated PucC protein disrupts LH1 assembly, pointing towards a PucC dimeric or multimeric functional unit.

Keywords Photosynthesis · Purple bacteria · Light-harvesting · LH1 · LH2 · *Rhodobacter* · PucC · LhaA · Bacteriochlorophyll delivery homologues · Complex-specific assembly factors

Abbreviations

BCD	Bacteriochlorophyll delivery
BChl	Bacteriochlorophyll *a*
ICM	Intracytoplasmic membrane system
LH1	Light-harvesting complex 1
LH2	Light-harvesting complex 2
RC	Reaction centre

Introduction

Purple photosynthetic bacteria such as *Rhodobacter capsulatus* contain light-harvesting complex 1 (LH1), which surrounds the reaction centre (RC) as part of the core complex, and light-harvesting complex 2 (LH2), which is present in multiple copies of a ring-shaped structure that interconnect core complexes (Bahatyrova et al. 2004; Papiz et al. 1996). These complexes are located within differentiated invaginations of the cytoplasmic membrane called the intracytoplasmic membrane system (ICM), which is formed in response to oxygen deprivation (Drews and Golecki 1995). The bacteriochlorophyll *a* (BChl) pigments of each LH complex have different Q_y (far-red) absorption maxima: BChl in the LH1 complex has a far-red

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absorption peak at ~875 nm, whereas the far-red absorption peaks of LH2 are at ~800 and 850 nm (Feick and Drews 1978).

LH1 and LH2 complexes consist of rings of transmembrane α and β proteins that bind carotenoid and BChl pigments, which absorb and transmit light energy to the RC complex (Cogdell et al. 1999). These LH complexes are absent from or present at a low level in cells that have mutations in genes called *lhaA* (for LH1) and *pucC* (for LH2). It is thought that maximal assembly of LH complexes does not occur spontaneously from proteins and pigments in vivo, but instead requires the complex-specific assembly proteins LhaA and PucC (Young and Beatty 2003).

The *Rba. capsulatus* PucC and LhaA proteins are 47% identical in alignments, and were found to have 12 transmembrane segments with an N-in/C-in membrane topology (Fig. 1). Other purple bacteria contain LhaA and PucC homologues as well as homologues of the *Rba. capsulatus orf428*, which is predicted to have the same membrane topology as PucC and LhaA (Young and Beatty 2003). Homologues of these bacterial sequences have been found in *Synechocystis PCC6803, Prochlorococcus marinus* and *Arabidopsis thaliana*. Thus there is a family of ~430 to 500 amino acid-long PucC-related proteins named the BChl delivery (BCD) family, which is grouped in the major facilitator superfamily of proteins (Saier et al. 1999). A tree of selected BCD proteins and homologues is shown in Fig. 2, with descriptions of the sequences in Table 1. This tree shows that the PucC, LhaA and *orf428*-related sequences each form separate clusters, with the plant and cyanobacterial homologues clustering more distantly.

The *Rba. capsulatus* PucC protein is encoded in a superoperon of *pucBACDE* genes (Fig. 3). A strong promoter drives transcription of *pucBA* genes (encoding LH2 α and β proteins), with transcription attenuated between *pucA* and *pucC* to yield large amounts of a *pucBA* message and lesser amounts of a *pucBACDE* message. The expression of

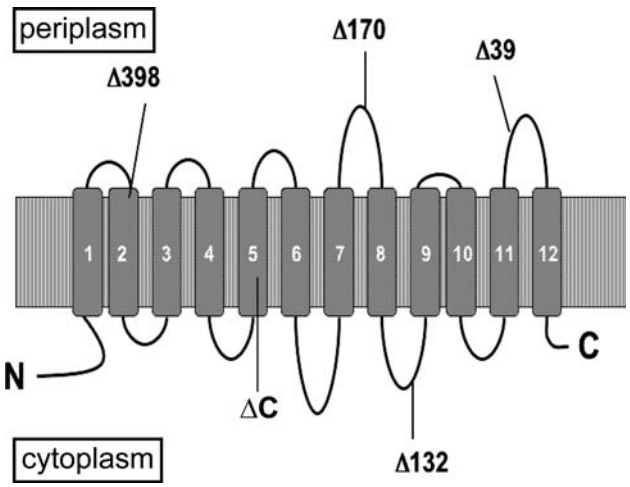

Fig. 1 Representation of *Rba. capsulatus* PucC protein membrane topology. A similar, 12-transmembrane topology was found for the *Rba. capsulatus* LhaA protein. Topologies are based on *phoA* and *lacZ* gene fusion analyses (LeBlanc and Beatty 1996; Young and Beatty 1998). The approximate positions of translationally in-frame *pucC*::*phoA* gene fusions are indicated: Δ398, fusion lacks the C-terminal 398 amino acids of PucC; Δ170, fusion lacks the C-terminal 170 amino acids of PucC; Δ132, fusion lacks the C-terminal 132 amino acids of PucC; Δ39, fusion lacks the C-terminal 39 amino acids of PucC. ΔC, location of the C-terminal 269 amino acid truncation of PucC in the pΔBADE and pΔC plasmids

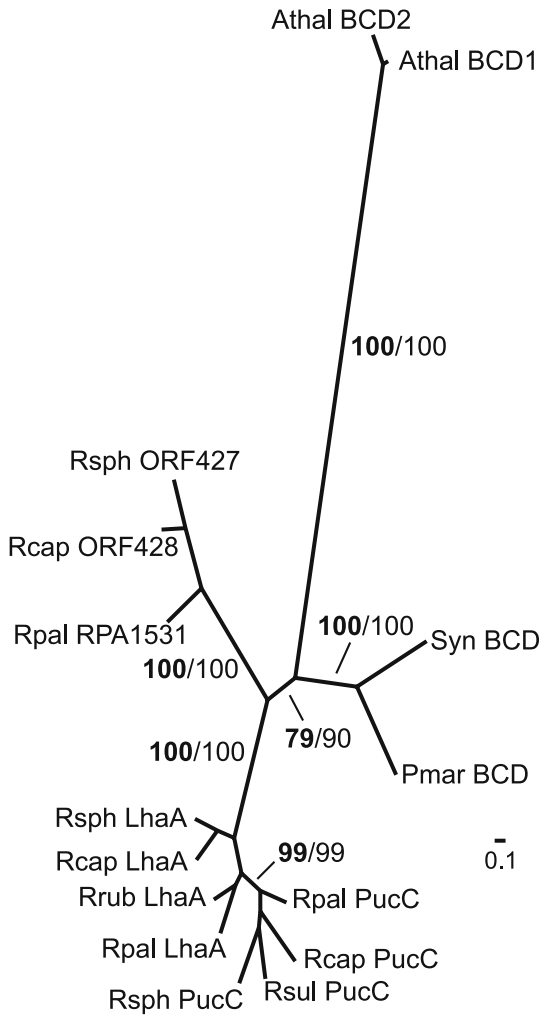

Fig. 2 Unrooted tree of BCD proteins and homologues. Abbreviations are: Athal, *Arabidopsis thaliana*; Pmar, *Prochlorococcus marinus*; Rcap, *Rhodobacter capsulatus*; Rpal, *Rhodopseudomonas palustris*; Rrub, *Rhodospirillum rubrum*; Rsph, *Rhodobacter sphaeroides*; Rsul, *Rhodovulvum sulfidophilum*; Syn, *Synechocystis sp.* PCC 6803. The unrooted Bayesian maximum likelihood tree is shown, with Bayesian clade credibility values (based on 100,000 generations) labelled in bold at the major branches followed by bootstrap values from the neighbour-joining analysis (percentages based on 10,000 replicates). The Bayesian scale bar is shown. Identification information and accession numbers for the sequences are given in Table 1

Table 1 Identification information for BCD proteins

Species	Protein	Locus name	Length (aa)	Accession
Rhodobacter capsulatus	PucC	*pucC*	461	P23462
Rhodobacter capsulatus	LhaA	*lhaA*	477	P26176
Rhodobacter capsulatus	ORF428	*orf428*	428	P26171
Rhodobacter sphaeroides	PucC	RSP_0315	459	NC_007493
Rhodobacter sphaeroides	LhaA	RSP_0290	479	NC_007493
Rhodobacter sphaeroides	ORF427	RSP_0278	427	NC_007493
Rhodopseudomonas palustris	PucC	RPA1493	478	NC_005296
Rhodopseudomonas palustris	LhaA	RPA1547	477	NC_005296
Rhodopseudomonas palustris	RPA1531	RPA1531	438	NC_005296
Rhodospirillum rubrum	LhaA	Rru_A0618	480	NC_007643
Rhodovulvum sulfidophilum	PucC	*pucC*	454	P95656
Prochlorococcus marinus 9211	BCD	P9211_07147	472	ZP_01005961
Synechocystis sp. PCC 6803	BCD	sll1906	484	NP_441204
Arabidopsis thaliana	BCD1	AT5G25040	434	NP_197886
Arabidopsis thaliana	BCD2	AT5G25050	499	NM_122414

pucE is boosted by a promoter within *pucD* (LeBlanc and Beatty 1993). The function of *pucD* and *pucE* are unknown, although the 14 kDa PucE protein (also known as LH2 γ) co-purifies with the LH2 complex (Tichy et al. 1989), and deletion of *pucE* reduces the amount of LH2 (LeBlanc and Beatty 1993).

In this article, we report on molecular genetic approaches to explore functions of the 461 amino acid PucC protein. It was found that a deletion of 269 codons from the 3′ end of the *pucC* gene reduces LH1 levels, but only when the *pucBA* genes are also present. Expression of truncations of the C-terminal region of PucC in *trans* to this *pucC*-deleted *puc* operon were found to progressively decrease LH1 levels as greater amounts of the C-terminal region were removed. We present a speculative model in which the PucC protein forms a dimer or multimer that has a shepherding activity, which prevents mixing of LH2 α and β proteins with the homologous LH1 α and β proteins, to allow optimal assembly of LH1.

Materials and methods

Bacterial strains, plasmids, growth conditions and absorption spectroscopy

The *Rba. capsulatus* strain ΔLHII (containing a chromosomal deletion of the *puc* operon) was described previously (LeBlanc and Beatty 1993). The pRK415-based (Keen et al. 1988) plasmids pΔC and pΔCDE were described previously (LeBlanc and Beatty 1996). Briefly, pΔC carries a *puc* operon in which the *pucC* gene is truncated at codon 192 (see Fig. 1), while the *pucBADE* genes are intact.

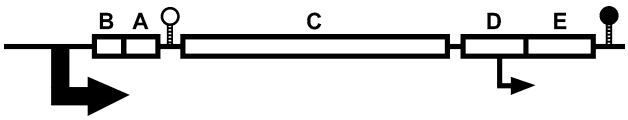

Fig. 3 Representation of the *Rba. capsulatus pucBACDE* superoperon. The relative strength of the two promoters is indicated by the sizes of the bent arrows, a transcription attenuator is represented by the empty stem-loop, and the transcription terminator is represented by the filled stem-loop. See LeBlanc and Beatty (1993) for details

Plasmid pΔCDE contains a deletion extending from *pucC* codon 192 through the stop codon of *pucE*, leaving *pucBA* and the N-terminal 192 codons of *pucC* as in pΔC. Empty control plasmids pRK415::Ω (LeBlanc and Beatty 1993) and pPUFQ (Bollivar et al. 1994) were described previously. The construction of plasmids pPQ::C and pPUFQ derivatives containing *pucC::phoA* fusions has been described (LeBlanc and Beatty 1996).

Cultures were grown semiaerobically in flasks containing RCV medium (Beatty and Gest 1981) as described (Aklujkar and Beatty 2006).

Intact cells of *Rba. capsulatus* were suspended in 24% bovine serum albumin and scanned in a spectrophotometer as described previously (Aklujkar and Beatty 2006).

Phylogenetic analyses

Sequences were aligned with Clustal X v1.81 (Thompson et al. 1997) and the alignment was used for Bayesian analysis with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2003) and neighbour-joining analysis using PAUP* v4.0 (Swofford 2000).

Results and discussion

Co-expression of a *pucC* deletion and *pucBA* genes reduces the amount of LH1

The *pucBACDE*-deleted strain ΔLHII lacks LH2, and so the absorption spectrum of cells has a major far-red peak at 875 nm (LH1) and smaller RC peaks at 800 and 760 nm (Fig. 4, top). Complementation of strain ΔLHII with a plasmid containing the entire *puc* operon restores the LH2 complex (LeBlanc and Beatty 1993).

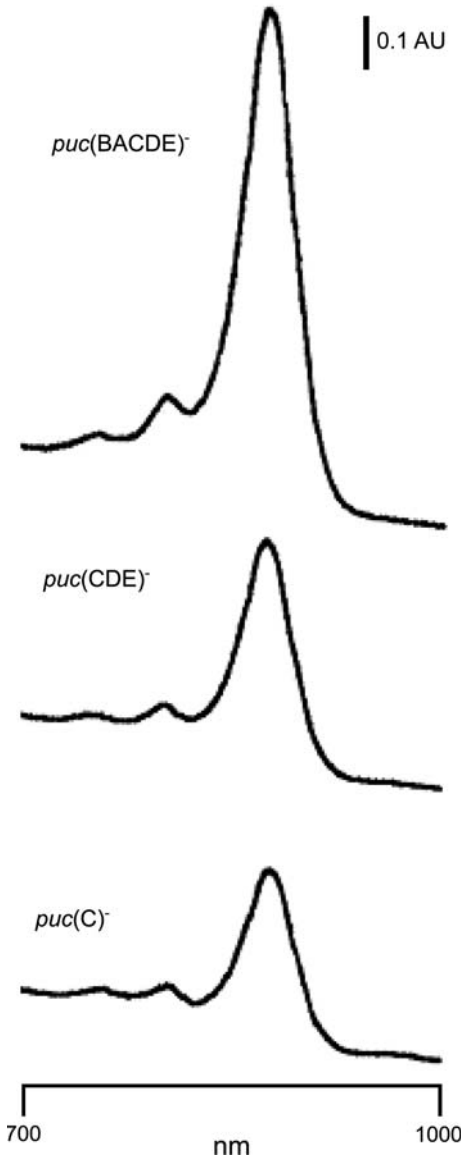

Fig. 4 Absorption spectra of ΔLHII intact cells containing plasmids expressing various *puc* operon genes. Top trace, ΔLHII containing empty vector pRK415::Ω; middle, ΔLHII containing plasmid pΔCDE; bottom, ΔLHII containing plasmid pΔC. Cultures were grown to similar cell density under low aeration in the absence of illumination, with spectra normalized to light-scattering at 650 nm

When plasmid pΔCDE, which encodes only PucB, PucA and the N-terminal 192 amino acids of PucC, was introduced into ΔLHII the LH1 peak was reduced to 47% of the amplitude seen when the empty vector pRK415::Ω was present (Fig. 4, compare top and middle traces). This shows that the expression of *pucBA* genes in the absence of *pucCDE* interferes with the accumulation of LH1. When plasmid pΔC, which expresses a *puc* operon containing an internal deletion of 269 codons from the 3′ end of *pucC* (leaving the N-terminal 192 codons), was introduced into strain ΔLHII the LH1 peak was reduced to 38% of the amplitude seen when the empty vector pRK415::Ω was present (Fig. 4, compare top and bottom traces).

These data indicate that the expression of *pucBADE* in the absence of the full-length *pucC* gene decreases the amount of LH1 to a greater extent than similarly truncated PucC in the absence of *pucDE*. From these results we suggest that the PucC protein has a shepherding activity that prevents the mixing of LH2 α and β proteins with the homologous LH1 α and β proteins during LH1 complex assembly, and lack of this activity seems to be more detrimental to LH1 assembly in the presence of *pucDE*.

Trans-expression of PucC N-terminal segments exacerbates the *pucC* deletion effect on LH1 levels

In studies of PucC membrane topology, translationally in-frame fusions between different regions of the *pucC* gene and the *E. coli phoA* gene were created and found to produce hybrid proteins (LeBlanc and Beatty 1996). As represented in Fig. 1, these fusions result in progressively shorter N-terminal segments of PucC, all fused to the same position of the PhoA protein. These fusions were exploited to investigate whether the shepherding function of PucC could be localized to a specific region of this protein. The experiments were done by expressing each of the *pucC::phoA* fusions in the ΔLHII (pΔC) strain, using a second plasmid (based on pPUFQ) that is compatible with pΔC.

As shown in Fig. 5a, expression of the Δ39 fusion protein lacking the C-terminal 39 amino acids of PucC did not restore LH2, in contrast to full-length *pucC* which was expressed from pPQ::C. The requirement for C-terminal amino acids for LH2 assembly confirms previous results which found that translationally out-of-frame fusions lacking the last transmembrane segment of PucC did not restore LH2 (LeBlanc and Beatty 1996). The Δ39 fusion protein did not affect the LH1 absorption peak, as compared to the LH1 peak of ΔLHII containing the pΔC or

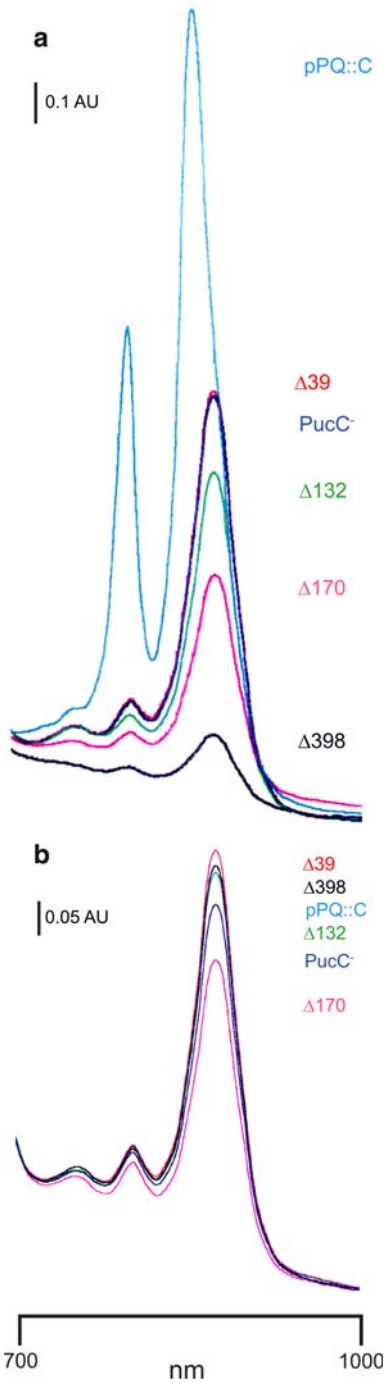

Fig. 5 Absorption spectra of ΔLHII intact cells containing plasmids expressing C-terminal *pucC*::*phoA* fusions. (**a**) cells containing plasmid pΔC, co-resident with a second plasmid listed below. Spectral traces: *turquoise*, plasmid pPQ::C (expressing full-length *pucC*); *red*, pΔ39; *blue*, empty *phoA* fusion vector pPUFQ; *green*, pΔ132; *pink*, pΔ170; *black*, pΔ398. See Fig. 1 for truncation locations in membrane topology of PucC protein. Cultures were grown under low aeration in the absence of illumination, to the same cell density, and spectra were normalized to light-scattering at 650 nm. (**b**) cells containing only *pucC*::*phoA* fusion plasmids; colour-coding the same as in part (**a**). Cultures were grown under low aeration in the absence of illumination, to the same cell density, and spectra were normalized to light-scattering at 650 nm

empty vector pPUFQ plasmids. Therefore, the presence of a PucC::PhoA fusion protein per se does not affect LH1 accumulation.

The Δ132 fusion of PucC to PhoA, which removes 132 residues from the C-terminus, resulted in an LH1 peak height 81% of the controls that contained the pΔC or empty vector pPUFQ plasmids (Fig. 5a). The Δ170 fusion removing 170 C-terminal residues yielded 56% of the control LH1 peak amplitude, and removal of 398 residues resulted in 16% of the controls.

The interpretation of these results is that the proposed shepherding activity of PucC appears to be spread over the central to N-terminal region of the protein. The progressively lower amounts of LH1 seen as the *pucC::phoA* fusion position was moved from the C-terminal to the N-terminal region of PucC are thought to be due to interference with residual shepherding activity of the 192 amino acid N-terminal segment of the PucC protein encoded by the pΔC plasmid.

To evaluate whether the *pucC::phoA* fusions alone have a direct effect on LH1 accumulation in the absence of pΔC, the plasmids expressing these fusions were introduced into strain ΔLHII. As shown in Fig. 5b, there was little or no effect on LH1 levels in these cells, and so the relatively strong effect of the PucC::PhoA fusion proteins on LH1 assembly must be due to the presence of the *pucBADE* and truncated *pucC* genes expressed from pΔC.

Thus it appears that there are multiple components of PucC that may function cooperatively, perhaps including an interaction between PucC proteins in the cell. The exact function(s) of the BCD family of proteins is not known. In fact, the name "BChl delivery" was based on a combination of experiments on the *Rba. capsulatus* LhaA protein that showed a decreased rate of LH1 assembly in an *lhaA* mutant, sequence similarities between BCD proteins, and similar membrane topologies of PucC and LhaA proteins relative to major facilitator superfamily proteins that were shown to transport small molecules across the cell membrane (Saier et al. 1999). The results described in this article are consistent with a speculative model in which a function of the PucC protein is to shepherd or sequester LH2 α and β proteins, as well as delivering BChl to nascent LH2 complexes. The varying amounts of LH1 resulting from the expression of different *pucC::phoA* gene fusions in the presence of pΔC, and the absence of such an effect in the absence of pΔC, argues for interaction of PucC proteins as a dimer or multimer. It would be interesting to evaluate the phenotype of plant (*A. thaliana*) and cyanobacterial (*Synechocystis sp.* PCC 6803) knockouts of BCD-homologous genes, to see if comparison to our work on *Rba. capsulatus* results in the emergence of a unifying model for the function of these widely conserved proteins.

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