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The photosynthetic deficiency due to *puhC* gene deletion in *Rhodobacter capsulatus* suggests a PuhC protein-dependent process of RC/LH1/PufX complex reorganization

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Abstract

Optimal photosynthetic reaction centre (RC) and core antenna (LH1) levels in the purple bacterium *Rhodobacter capsulatus* require the *puhC* gene. Deletion of *puhC* had little effect on RC and LH1 assembly individually, but significantly inhibited the photosynthetic growth of $RC^{+}LH1^{-}$ strains, suggesting that maximal RC catalytic activity is PuhC-dependent. Consistent with post-assembly reorganization of the RC/LH1/PufX core complex by PuhC to include latecomer proteins, spatial separation of *pufX* from the RC/LH1 genes inhibited PufX accumulation and photosynthetic growth only in $PuhC^{-}$ strains. Photosynthetic activity improved to different degrees when PuhC homologues from three other species were expressed in $PuhC^{-}$ *R. capsulatus*, indicating that PuhC homologues function similarly but may interact inefficiently with a heterologous core complex. Anaerobic photosynthetic growth of $PuhC^{-}$ strains was affected by the duration of prior semiaerobic growth, and by two genes that modulate bacteriochlorophyll production: *pufQ* and *puhE*. These observations agree with a speculative model in which reorganization of the core complex is an important regenerative process, accelerated by PuhC.

Keywords: Photosynthesis; Purple bacteria; Light-harvesting; Reaction center; *Rhodobacter*; Bacteriochlorophyll

Purple nonsulfur photosynthetic bacteria such as *Rhodobacter capsulatus* are capable of aerobic respiratory and anaerobic photosynthetic growth. The photosynthetic apparatus includes three transmembrane pigment–protein complexes: the reaction centre (RC),[1] where light-dependent electron transfer is initiated; light harvesting (LH) complex 1, which forms a ring or arc encircling the RC as part of the so-called core complex [1–13]; and the LH2 complex, which is present in multiple copies of a ring-shaped structure that interconnects core complexes [2,14]. These complexes are located within differentiated invaginations of the cytoplasmic membrane called the intracytoplasmic membrane system (ICM), formed in response to oxygen deprivation [15]. The presence of photosynthetic complexes can be evaluated by their characteristic near-infrared light absorption spectra, which reflect the protein environments around bacteriochlorophyll *a* (BChl). Unbound BChl has an absorption peak at 780 nm, whereas the peaks of the LH2 BChls of *R. capsulatus* are at 800 and 850 nm, and BChl in the LH1 complex absorbs light of approximately 880 nm [16]. The near-infrared absorption peaks of the RC are at about 760 nm (bacteriopheophytins; BPhe), 804 nm (accessory or 'voyeur' BChls), and 865 nm (the 'special pair' of BChls) [16].

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[1] *Abbreviations used:* RC, reaction centre; ICM, intracytoplasmic membrane system; BChl, bacteriochlorophyll *a*; LH, light harvesting; GTA, gene transfer agent; LB, Luria–Bertani.

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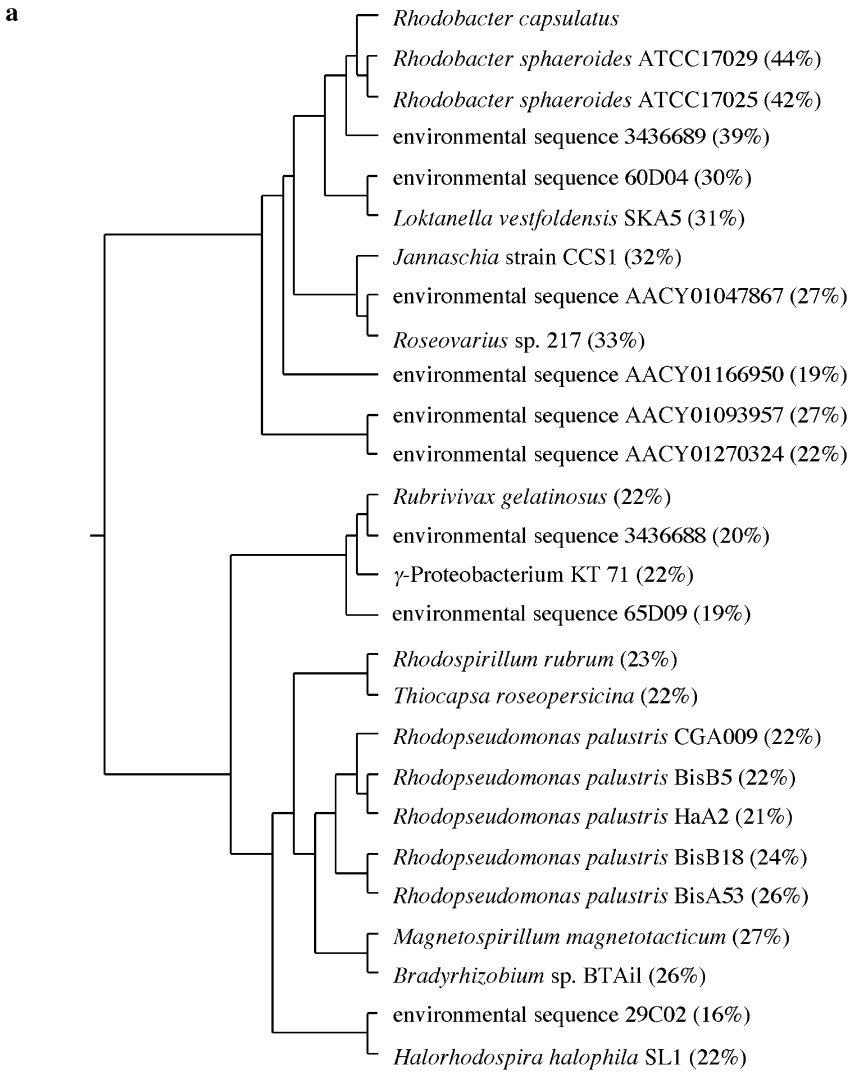
In *R. capsulatus*, two of the three RC proteins, called RC L and RC M, both polypeptides of LH1 (LH1 α and LH1 β), and the associated protein PufX are encoded by the *pufQBALMX* operon [17]. The *pufQ* gene product stimulates BChl production [18–20]. The remaining RC protein, RC H, is encoded by the *puhA* gene that is transcribed as part of the *bchFNBHLM-lhaA-puhABCE* superoperon from two promoters, one 5′ of *bchF* and the other within the *lhaA* gene [21–23]. The PuhB protein is an RC assembly factor [24,25], PuhC (formerly known as Orf162b) is required for optimal RC/LH1 levels and photosynthetic growth [21], and PuhE is a negative modulator of BChl production [18]. The exact function of PuhC has not been established, nor have genes similar to *puhC* (Fig. 1a) been



b predicted cytoplasmic transmembrane periplasmic domain

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                        X X X   X        X  *    X     X   X       X X    X            X    *
1. --MAQLPLSPAPQRPETKTPGKPEAELIPKPLLRAMIGIALLSLALTTYAVLTGRPHEGVPAPGKVVAEKLVVLKDIDARHATVSDPE-GNIL
2. ----------MSAQNSRQPRSDKDKELIPPFLLKAMFALALGSVLMVSWAVWTGREPTGKPAPAPVVAERQLVLQGLGEQAVAVRTPE-GETL
3. ------------------MSGEVRHIRRVSDRLMALIALGLFFVVAAVLLARTTDNIIVAEPPVGSLLQGKILLFNDGPNGEVAISDEATGAVV
4. ------------------MSAGHRDPIFPRGLKIATLGLVLLTFGLIGFSRLTDVGHSTLP-AATAEGSAWISFLPRENGDVAVVERDSAREI

           ***        * *          X *X   ** *X* *  * X  X* *** X*  X* X*X
1. LDLP--EGGFVDVMAAAVRRSRAVARITDNPPVRIVRYDNGRLAMEDPATGWSTELYAFGADSKAAFERILDMK
2. FEAE--NGGFVTVIQIGLKRARTVHRIEGNPPVRLVKYENGRLSLQDDATGWSAELQAFGPDNEAAFERMLSE
3. RTLYAGEGSFIRGVVRSLVRTRHQQGLFAQTGFHLNLYEDGRLQLVDPLTSQVIDLVAFGPTNMAEFAGLLTLEPGGALMADGT
4. AILASGDNNFAVGMLRGLARQRARIGVAATDPYELTRWTDGRLTMTDPATGHIIVANAFGSKSAAQMNGFYDAAQAARDGR
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Fig. 1. Evolutionary relationships of predicted *puhC* gene products. (a) Phylogenetic tree of PuhC proteins, plotted with ClustalW software (http://www.es.embnet.org). The percentage identity of each sequence to the *R. capsulatus* PuhC protein is given in parentheses. (b) Sequence alignment of four *puhC*-like sequences from (1) *R. capsulatus*; (2) *R. sphaeroides* ATCC17029; (3) *R. gelatinosus*; and (4) *R. rubrum*. Asterisks mark aminoacyl residues that are identical or similar in >80% of the PuhC sequences in (a), and "X" marks residues that are identical or similar in all PuhC proteins of *pufX*-containing (*Rhodobacter*) species.