The *psbC* start codon in *Synechocystis* sp. PCC 6803

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The translation start codon for *psbC*, the gene encoding CP43, a chlorophyll-binding protein of photosystem II, has been identified for the cyanobacterium *Synechosystis* sp. PCC 6803 using site-directed mutagenesis. An AUG codon, about 50 bases upstream from the end of *psb*D-I had previously been assumed to be the translation start site of *psb*C. However, the fact that the AUG codon is not present in *psb*C from several other organisms, whereas a GUG codon 14 bases upstream from the end of *psb*D-I is strictly conserved suggests that CP43 translation starts at the latter codon. Mutation of GUG, but not of AUG, led to a loss of CP43 and photoautotrophic growth, indicating that the GUG codon is the sole initiation site for translation of the CP43 protein in *Synechocystis* sp. PCC 6803.

Gene, *psb*C; Translation initiation; CP43; Cyanobacterium; Photosystem II

1. INTRODUCTION

Photosystem II (PS II) is composed of at least 6 integral membrane proteins forming a core complex, as well as several peripheral polypeptides [1]. The PS II core proteins are highly conserved in structure and function between higher plants and cyanobacteria [2]. These core proteins include two chlorophyll-binding proteins, CP43 and CP47, two putative reaction center proteins, D1 and D2, cytochrome b559, and the gene product of *psb*I [3] the function of which is still unknown. The CP43 protein, which is involved in the transfer of energy from the bound antenna pigments to the reaction center, is encoded by *psb*C, a single-copy gene in both plants and cyanobacteria, which partially overlaps with *psb*D in cyanobacteria and higher plants (but not in certain green algae). In cyanobacteria, there are two copies of *psb*D, *psb*D-I and *psb*D-II, one of which, *psb*D-I, is cotranscribed with *psb*C.

Putative start codons for CP43 include an AUG 50 nucleotides upstream from the end of *psb*D-I and a GUG 14 bases from the end of *psb*D-I [4]. Since the GUG codon is conserved in all species in which *psb*C has been sequenced, including higher plants [5–7], cyanobacteria [4,8] and *Chlamydomonas reinhardtii*

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Abbreviations: CP43, 43 kDa chlorophyll-binding protein of PS II; PS II, photosystem II; bp, base pairs

[9], whereas AUG is not found in *Chlamydomonas* and a cyanobacterium, *Synechococcus* sp. PCC 7942 [8], the presumption is favored that GUG may be the translation start codon in CP43. The N-terminal sequence of mature CP43 in higher plants starts with a Thr encoded by the second codon behind the GUG [10], which is compatible with either codon being the potential start site.

Synechocystis sp. PCC 6803 has proved invaluable as an organism of study for the elucidation of the function of PS II proteins by specific mutagenesis (reviewed in [11]). Its photosynthetic apparatus is comparable to that of higher plants, and its genes can be manipulated quite easily [12]. Utilizing site-directed mutagenesis to alter either of the putative start codons, we were able to positively identify the translation initiation site of the CP43 protein.

2. MATERIALS AND METHODS

Synechocystis sp. PCC 6803 was cultured as previously described [13,14]. Culture doubling time was determined as in [14]. Standard cloning techniques [15] were used to construct *E. coli* plasmids carrying cyanobacterial genes. Oligonucleotide-directed mutagenesis of the *psb*D-I/C operon was done as described in [16,17]. The oligonucleotides used were 5′ AGTTTTC<u>G</u>TGGGGTT 3′, 5′ AGTTTTC<u>TT</u>GGGGTT 3′, 5′ CGTTACC<u>GC</u>GGGGGA 3′, and 5′ GCGTTAC<u>TA</u>CGGGGGA 3′ for ATG > ACG, ATG > AAG, GTG > GCG, and GTG > GTA mutations, respectively. They are complementary to the appropriate regions of the wildtype *psb*D-I/C operon except at the underlined nucleotide. Subsequent mutant identification and plasmid construction procedures have been described in [18]. The creation of a double-deletion mutant of *Synechocystis* sp. PCC 6803 lacking the *psb*D-I/C operon as well as *psb*D-II, in order to introduce the mutated *psb*D-I/C operon into *Synechocystis* and obtain *psb*C expression solely from the mutated gene has also been described in [18]. Herbicide binding experiments, Western blotting,

and immunodetection (using BCIP (5-bromo-4-chloro-3-indolyl phosphate, p-toluidine salt)/NBT (p-nitro blue tetrazolium chloride) staining to detect alkaline phosphatase linked to the secondary antibody) were done according to previously published procedures [19,20].

3. RESULTS AND DISCUSSION

In *Synechocystis* sp. PCC 6803 there are two potential start sites of *psb*C translation: the AUG codon 50 nucleotides upstream from the end of *psb*D-I, and the GUG codon 14 bases upstream from the end of *psb*D-I [4] (fig.1). It has been noted that the presumed ribosome-binding sequence preceding the GUG codon has a higher degree of similarity to the Shine-Dalgarno consensus sequence than the AUG codon [4]. However, a higher degree of similarity does not necessarily imply a higher ribosome affinity [21].

To determine which codon(s) can be used to initiate CP43 translation, site-directed mutations were introduced into these codons using our previously published mutagenesis strategy [18]. Four *Synechocystis* mutants were generated utilizing in vitro site-directed mutagenesis and subsequent transformation of the appropriate deletion mutant with the altered *psb*C genes (table 1). The site-specific changes in the presumed start codons of *psb*C also affected the sequence of the overlapping *psb*D-I. However, when possible, mutations were made such that they did not affect the D2 amino acid sequence. In addition, in the case of the GUG > GCG, GUG > GUA, and AUG > ACG mutations, the modified genes were also introduced into a *psb*D-I/C deletion mutant where the *psb*D-II gene can supply copies of the unaltered D2 protein in the mutant. Thus, any loss in PS II activity in such mutants must be due to a change in CP43 rather than to one in the D2 protein. All mutants in which the AUG codon was changed grew photoautotrophically (with PS II activity to support growth), while the mutants having a change at the GUG site could only grow photoheterotrophically (indicating the loss of PS II activity) (table 2). Photoautotrophic growth in the GUG mutants could be restored by transformation with a 0.4 kbp wild-type *psb*D-I/C fragment covering the *psb*C start region, indicating that no mutations leading to a change in PS II activity have occurred in other regions.

Table 1

Site-directed mutations in the putative start codons of CP43 and the resulting phenotypes

Codon change	a.a. change in CP43	a.a. change in D2	PS II activity	Presence of CP43 in the thylakoid
AUG>ACG	M-1-T	H-336-H	+	+
AUG>AAG	M-1-K	H-336-Q	+	+ (d.n.s.)
GUG>GCG	M/V-13-A	R-348-R	−	−
GUG>GUA	M/V-13-V	G-349-S	−	−

See fig.1 for the locations of the mutations. Note that the amino acid coded by GUG is Met if GUG is the start codon, and Val if CP43 translation initiates at AUG. d.n.s., data not shown

Table 2

Growth rates (doubling times) of various strains of *Synechocystis* sp. PCC 6803 under photoautotrophic conditions (in BG-11 medium without glucose) and photoheterotrophic conditions (in BG-11 medium with 5 mM glucose)

Strains	Doubling time (h)	
	BG-11 without glucose	BG-11 with glucose
Wild type	13	11
AUG>AAG	13	11
AUG>ACG	13	12
GUG>GCG	∞	13
GUG>GUA	∞	13

The presence of CP43 in the thylakoids of the GUG mutants and of one of the AUG mutants was probed using antisera raised against spinach CP43. In wild type and the AUG mutant, but not in the GUG mutants, CP43 was detected (fig.2). Herbicide binding analyses using radiolabeled diuron revealed no significant diuron binding in cells of GUG mutants (data not shown), as is also found in *psb*C interruption mutants [20]. These results, therefore, unequivocally identify the GUG codon located 14 nucleotides upstream of the end of *psb*D as the start codon for CP43 in *Synechocystis* sp. PCC 6803. Our observations also indicate that upon inactivation of the GUG start codon, the AUG codon cannot serve as an alternative translation initiation site: in thylakoids from the GUG mutants no CP43 can be detected.

It may be interesting to note that *E. coli* containing a plasmid carrying the entire *psb*D-I/C operon is slow-
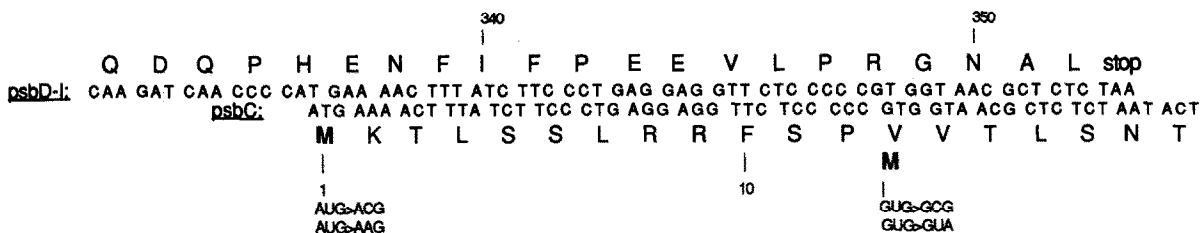

Fig.1. Protein and nucleotide sequences of the region in *Synechocystis* sp. PCC 6803 including the end of *psb*D-I (encoding the D2 protein) and the beginning of *psb*C (encoding the CP43 protein). Site-directed mutations have been indicated. (Note: GUG as a start codon codes for *N*-formyl-Met.)
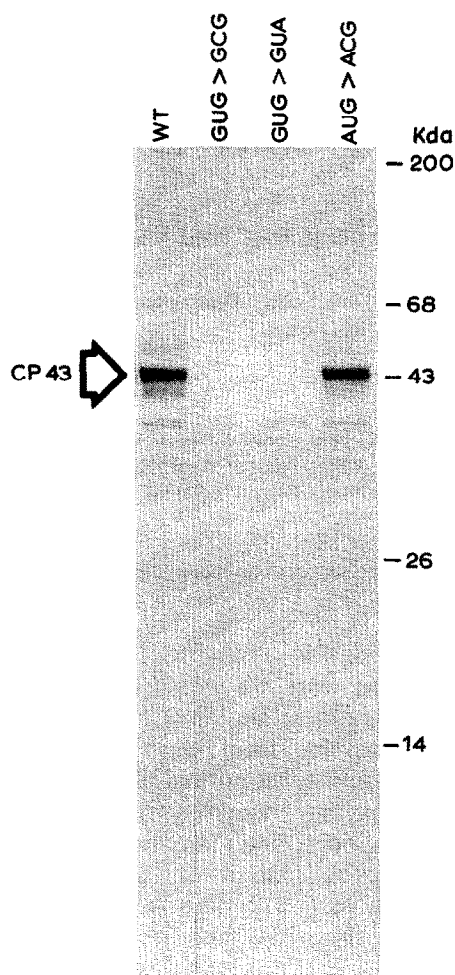
Fig.2. Immunoblot of thylakoid proteins from wild type (Wt) and site-directed *psbC* start codon mutants (base change as indicated), probed with antibodies against CP43. Wild type thylakoids and the AUG> ACG mutant contain the normal amount of CP43, while both GUG codon mutants completely lack CP43 in their thylakoids.

ed down considerably in its growth rate as compared to *E. coli* with a plasmid carrying another region of *Synechocystis* DNA. This is attributed to an expression of *psbC* in this bacterium which appears detrimental to its growth. However, *psbD*-I/C-carrying plasmids containing the mutations in the GUG codon did not interfere with normal *E. coli* growth (J. Charite, unpublished), suggesting that the *psbC* GUG start codon is also recognized as a start codon by the *E. coli* system. Mutations in the AUG codon did not alleviate slow growth in *E. coli* carrying the *psbD*-I/C-containing plasmid.

It remains an open question why the CP43 translation start site is GUG, whereas that of the other large PS II core components is AUG. One of the potential reasons for this may reside in the different turnover rates of the two proteins encoded in the *psbD*-I/C operon: since D2 is thought to have a relatively high degradation rate as compared to CP43 in the light [22], less translation of *psbC* is needed as compared to that

of *psbD*. Since the *N*-formyl-methionyl tRNA recognizes AUG about a factor of two better than GUG, the extent of initiation declines about half when AUG is replaced by GUG [23]. Thus, the cyanobacterial cell and the chloroplast may use this mechanism to differentiate the rates of synthesis of D2 and CP43, and thus establish a better balance between supply and demand for the two proteins.

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