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The primary structure of the γ-subunit of the ATPase from *Synechocystis* 6803

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The nucleotide sequence of the gene coding for the F_0F_1-ATPase γ-subunit (*atpC*) from the transformable cyanobacterium *Synechocystis* 6083 has been determined. The deduced translation product consists of 314 amino acid residues and is highly homologous (72% identical residues) to the sequences of other cyanobacterial γ-subunits. The *Synechocystis* 6803 sequence is also homologous to the chloroplast γ-sequence. Like in the other cyanobacterial subunits, only the first of the 3 cysteine residues, which are involved in energy-linked functions of the γ-subunit in spinach chloroplasts, is conserved in *Synechocystis* 6803.

F_0F_1-ATPase; γ-Subunit; Cyanobacteria; Nucleotide sequence; Amino acid sequence

1. INTRODUCTION

F_0F_1-ATPases (proton-translocating ATPase, EC 3.6.1.34) are multimeric enzyme complexes of the cytoplasmic membrane of eubacteria [1], the inner membrane of mitochondria, and the thylakoid membrane of cyanobacteria and chloroplasts [2,3]. Characterization by DNA sequence analysis of the genes has demonstrated that many features of their structures are conserved [4]. The data, however, also show that the subunits of the cyanobacterial ATP-synthase are more closely related to their equivalents from chloroplasts than to those of *Escherichia coli* and purple bacteria, in accordance with the anticipated common origin of cyanobacteria and chloroplasts.

The ATPase complex is composed of an oligomeric transmembrane sector (F_0) which acts as a proton channel, and a peripheral catalytic core (F_1) consisting of 5 different subunits (α, β, γ, δ and ε). The γ-subunit has been shown to be involved in light/dark regulation and thiol modulation of the chloroplast ATPase [5].

Here the primary sequence of the gene coding for the γ-subunit of F_1 from the transformable cyanobacterium, *Synechocystis* 6803 is reported. The open reading frame consists of 945 bases which encode a protein of 314 amino acids. It shows homologies with both the spinach [6] and the cyanobacterial [7,8] protein sequences, especially at the amino- and carboxyl-terminal ends. In variance to the 4 cysteines found in the spinach γ-subunit, only one cysteine residue could be detected in the deduced primary structure of the *Synechocystis* 6803 protein, in agreement with the two other cyanobacterial γ-subunits.

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The nucleotide sequence(s) presented here has (have) been submitted to the EMBL/GenBank database under the accession number Y07532

Abbreviations: DTT, dithiotreitol; NEM, *N*-ethylmaleimide; SDS, sodium dodecylsulfate

2. MATERIALS AND METHODS

2.1. *Cell growth and isolation of genomic DNA*

Synechocystis strain PCC 6803 was grown and cells were isolated as described by Dzelzkalns and Bogorad [9]. Genomic DNA was prepared according to the method of Murray and Thompson [10]. *E. coli* strain DH5α was used as the host for Bluescript KS M13+ plasmid (Stratagene cloning systems).

2.2. *DNA hybridization*

Restriction endonuclease digests of DNA were transferred from agarose gels to a nylon membrane (Pall Biodyne B) under alkaline conditions as described [11,12]. For prehybridization, the nylon filters were incubated at 37°C for 4 h in a solution containing 0.75 M NaCl, 0.075 M Na-citrate, 30% formamide, 0.1% SDS, 100 μg/ml herringsperm DNA, 0.1% (w/v) bovine serum albumin, 0.1% (v/v) Ficoll and 0.1% (v/v) polyvinylpyrrolidone. Hybridizations of the filters were carried out for 16–20 h in the presence of biotinylated 'random-primed' probes [13,14] at the same temperature and in the same solution as for prehybridization with 50 μg/ml herringsperm DNA instead of 100 μg/ml. The filters were then washed 2 times at 25°C for 10 min and 2 times at 52°C for 20 min in 0.3 M NaCl, 0.03 Na-citrate, 0.1% SDS, 2 times at 25°C for 10 min and 2 times at 52°C for 20 min. The biotin-labeled probe DNA-target DNA hybrids were detected using a streptavidin-alkaline phosphatase conjugate and the dyes Nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (BlueGene-system, Gibco-BRL) according to the manufacturer's instructions. A 1240 bp *Hpa*I fragment from the clone pAN703 (kindly provided by D.A. Bryant, Pennsylvania State University) encoding the *atpC* gene of *Anabaene* spec. PCC7120 [8] was used as hybridization probe. The 1240 bp *Hpa*I fragment was purified by electroelution from agarose gels. Biotin-labeled 'random-

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primed' probes were prepared as specified by the manufacturer (Boehringer, Mannheim) with biotin-11-dUTP (Gibco-BRL).

2.3. *Cloning and screening of the* Synechocystis atpC *gene*

Genomic DNA was digested with *Eco*RI and *Hin*dIII and separated on an agarose gel; DNA fragments in the range of 3.5–5.2 kbp were purified by electroelution from agarose gels. They were cloned in Bluescript plasmids. Screening of clones was carried out according to the colony hybridization method of Grunstein and Hogness [15] with radiolabeled (^{32}P]dATP, Amersham) 'random-primed' hybridization probes.

2.4. *Subcloning and DNA sequencing*

Nested deletions of a positive clone (pSyn1) were generated using an exonuclease III/mung bean nuclease system from Stratagene. Double-stranded DNA was sequenced by the dideoxy method [16] as modified by Tabor and Richardson [17,18] to incorporate [^{32}P]dATP using T7-polymerase from Pharmacia. Sequencing primers were supplied by Stratagene.

2.5. *Computer analysis*

DNA sequence analysis, translation algorithms and data bank search were conducted with the program *Fast a* [35] using the EMBL GenBank. Multiple alignments were carried out with the program *Clustal* [36].

3. RESULTS

The *atpC* gene of *Synechocystis* 6803 was found to be located on a 4.2 kbp *Eco*RI/*Hin*dIII fragment by Southern hybridization with the *atpC* gene of *Anabaena*7120. To isolate the gene, *Eco*RI/*Hin*dIII fragments between 3.5 and 5.2 kbp of *Synechocystis* 6803 genomic DNA were subcloned in Bluescript plasmids. One clone (pSyn1) with a 4.2 kbp insert containing the complete *atpC* gene of *Synechocystis* was identified by colony hybridization. The insert was further subcloned into a 870 bp *Eco*RI/*Bam*HI fragment containing 710 bp of the 5′-coding region and into a 1170 bp *Bam*HI/*Kpn*I fragment containing 235 bp of the 3′-region. These two fragments were used for sequencing. The nucleotide sequence (fig.1) was determined by sequencing a series of nested deletion clones. The gene contains 945 bp including the stop codon and encodes for 314 amino acid residues. A putative ribosome binding site can be localized 12 nucleotides upstream of the initiator ATG corresponding to a comparison of several genes from *Synechocystis* [19] where the Shine-Dalgarno sequences [20] are found, i.e. approximately 12–25 nucleotides upstream of the initiator codon. In the 3′-untranslated region, two inverted repeats of 9 nucleotides are found 24 nucleotides after the stop codon. They can be folded into a stem-loop structure that might be important for the stabilization of the respective mRNA (fig.1, [21]).

4. DISCUSSION

A comparison of the predicted amino acid sequence of the *Synechocystis* γ-subunit with those from other sources reveals high homologies at the amino as well as at the carboxyl-terminus (fig.2). The carboxyl-terminus was assumed to be important for the assembly of the F_1 complex [28]; it might be concluded that the amino-terminus likewise is involved in assembly of the complex.

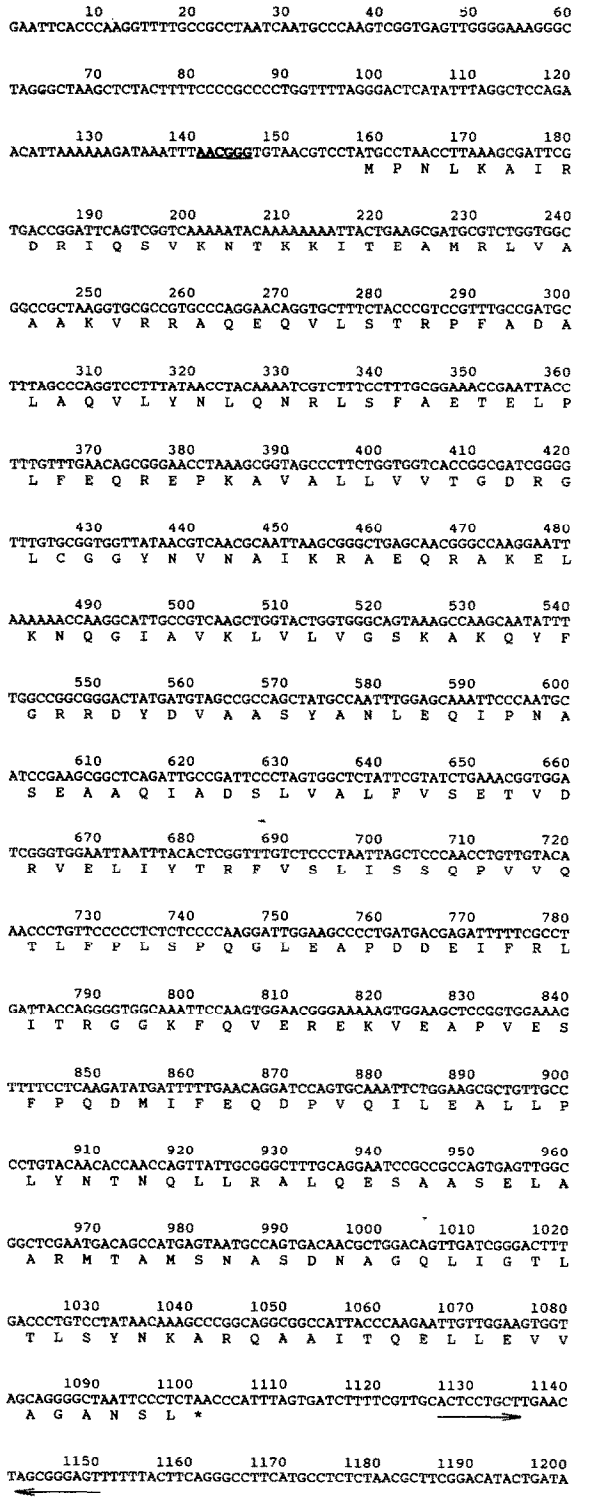

Fig.1. Nucleotide sequence of the *Synechocystis* 6803 *atpC* gene. The deduced translation product is shown below the nucleotide sequence. A potential ribosome-binding site is underlined, and inverted repeat sequences capable to form stem-loop structures in mRNA are marked with arrows.

The central region of the *Synechocystis* sequence shows less homology with γ-sequences of bacteria and mitochondria, but is very similar compared to other cyanobacterial and chloroplastic sequences. The overall identity between the amino acid sequences derived from the coding region of the *Synechocystis atpC* gene and those from other organisms varies between 35 and 75% (fig.3).

The sequence -DRGLCGG- (residues 86–92) is well conserved and contains a cysteine residue in all species except PS3[23]. In membrane-bound spinach ATPase, this cysteine (light-site cysteine, [24]) which is occluded in de-energized thylakoids gets accessible to NEM modification upon membrane energization. The involved light-induced conformational changes may play an essential role either in coupling between catalytic activity and the proton translocating activity or activation of the membrane-bound ATPase [5].

The region between residues 195–233 (fig.2) is conserved among cyanobacteria [7,8] and chloroplasts

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                    1
Synechocystis 6803  MPNLKAIRDR IQSVKNTKKI TEAMRLVAAA KVRRAQEQVL STRPFADALA
Synechococcus 6301  MANLKAIRDR IKSVRNTRKI TEAMRLVAAA KVRRAQEQVL STRPFADRLA
Anabaena 7120       MPNLKSIRDR IQSVKNTKKI TEAMRLVAAA RVRRAQEQVI ATRPFADRLA
cSpinacia oleracea  -ANLRELRDR IGSVKNTQKI TEAMKLVAAA KVRRAQEAVV NGRPFSETLV
cCh. reinhardtii    -G-LKEVRDR IASVKNTQKI TDAMKLVAAA KVRRAQEAVV NGRPFSENLV
E. coli             MAGAKEIRSK IASVQNTQKI TKAMEMVAAS KMRKSQDRMA ASRPYAETMR
Rp. blastica        MPSLKDLKNR IGSVKNTRKI TKAMQMVAAA KLRRAQDSAE AARPYAERMG
Rs. rubrum          MASLKDLRSR ITSVKSTQKI TSAMKMVAAS RLRRAQDTAE AARPYTQRME
PS3                 -ASLRDIKTR INATKKTSQI TKAMEMVLTS KLNRAEKREI V-RPYMEKIQ
mBos taurus         -ATLKDITRR LKSIKNIQKI TKSMKMVAAA KYARAERELK PARVYG----

                    51
Synechocystis 6803  QVLYNL-QNR LSFAETELPL FEQREPKAVA LLV-VTGDRG LCGGYNVNAI
Synechococcus 6301  QVLAGL-QQR LQFENVDLPL LQRREVKTVA LLV-VSGDRG LCGGYNSNVI
Anabaena 7120       QVLYGL-QTR LRFEDVDLPL LKKREVKSVG LLV-ISGDRG LCGGYNTNVI
cSpinacia oleracea  EVLYNM-NEQ LQTEDVDVPL TKIRTVKKVA LMV-VTGDRG LCGGFNNMLL
cCh. reinhardtii    KVLYGV-NQR VRQEDVDSPL CAVRPVKSVL LVV-LTGDRG LCGGYNNFII
E. coli             KVIGHLAHGN LEYKH---PY LEDRDVKRVG YLV-VSTDRG LCGGLNINLF
Rp. blastica        AVIASLASGQ G--AGAP-RL LAGNGRDQIH LLVVMTSERG LCGGFNSTIV
Rs. rubrum          RMLGNLAAST AGMAGAS-PL LGGTGKDNVH LIVALTANRG LCGGFNGSII
PS3                 EVVANVALA- ---ARASHPM LVSRPVKKTG YLV-ITSDRG LAGAYNSNVL
mBos taurus         --VGSLAL-- --YEKADI-- -KTPEDKKKH LIIGVSSDRG LCGAIHSSVA

                    101
Synechocystis 6803  KRAEQRAKEL KNQGIAVKLV LVGSKAKQYF GRRDYDVAAS YANLEQIPNA
Synechococcus 6301  RRAEQRAREL SAQGLDYKFV IVGRKAGQYF QRREQPIEAT YSGLEQIPTA
Anabaena 7120       RRAENRAKEL KAEGLDYTFV IVGRKAEQYF RRREQPIDAS YTGLEQIPTA
cSpinacia oleracea  KKAESRIAEL KKLGVDYTII SIGKKGNTYF IRRPEIPVDR YFDGTNLPTA
cCh. reinhardtii    KKTEARYREL TAMGVKVNLV CVGRKGAQYF ARRKQYNIVK SFSLGAAPST
E. coli             KKLLAEMKTW TDKGVQCDLA MIGSKGVSFF NSVGGNVVAQ VTGMGDNPSL
Rp. blastica        RLARQRANEL VAQGKTVKIL TVGKKGREQL KRDWASAFVG HVDLSDVRRL
Rs. rubrum          RATRTLVREL EAQGKTVKLL CIGKKGRDGL KREFPKQIIG GIADQSSKAI
PS3                 RLVYQTIQKR HASPDEYAII VIGRVGLSFF RKRNMPVILD ITRLPDQPS-
mBos taurus         KQMKSEAANL AAAGKEVKII GVGDKIRSIL HRTHSDQFLV TFKEVGRRPP

                    151
Synechocystis 6803  --SEAAQIAD SLVALFVSET VDRVELIYTR FVSLISSQPV VQTLFPLSPQ
Synechococcus 6301  --QEANDIAD ELLSLFLSGT VDRVELVYTK FLSLVASNPV VQTLLPLDPQ
Anabaena 7120       --DEANKIAD ELLSLFLSEK VDRIELVYTR FVSLVSSRPV IQTLLPLDTQ
cSpinacia oleracea  --KEAQAIAD DVFSLFVSEE VDKVEMLYTK FVSLVKSDPV IHTLLPLSPK
cCh. reinhardtii    --KEAQGIAD EIFASFIAQE SDKVELVFTK FISLINSNPT IQTLLPMTPM
E. coli             --SELIGPVK VMLQAYDEGR LDKLYIVSNK FINTMSQVPT ISQLLPLP--
Rp. blastica        GYSNAQGIAR EVLAAFEAGE ADVVTIFYNR FQSVISQVPT AQQVIPAK--
Rs. rubrum          GFSDADRFSR LILDMFQAGE FDVCTLVYNR FQSAISQVVT RQQIIPFAVP
PS3                 -FADIKEIAR KTVGLFADGT FDELYMYYNH YVSAIQQEVT ERKLLPLT--
mBos taurus         TFGDASVIAL ELLN--SGYE FDEGSIIFNR FRSVISYKTE EKPIFSLDT-
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Fig.2. Alignment of the protein sequences of the γ-subunits of ATPases from cyanobacteria [7,8], chloroplasts (c, [6,32]), bacteria [23,25–27] and mitochondria (m, [28]). Identities are boxed and gaps are marked by insertion of broken lines. The total number of amino acids (aa) of the various proteins is given at the end of the sequences.

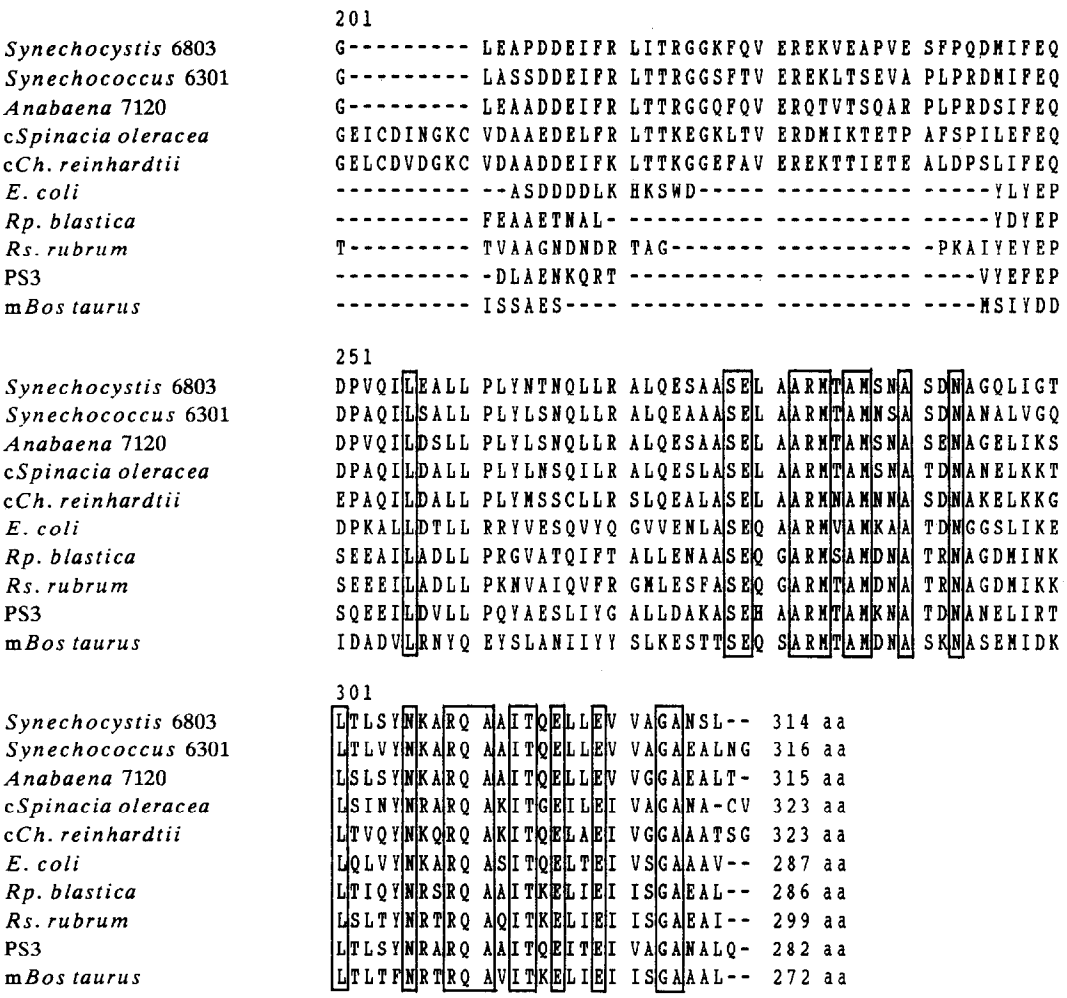

Fig.2. (continued)

[6,32] but is absent in photosynthetic (non-sulfur) [25,26] and other bacteria [23,27] and in mitochondria [28]. It contains the first of two trypsin cleavage sites [29]; cleavage between the spinach residue Arg-215 and Leu-216 (corresponding to *Synechosystis* residues 207 and 208) was shown to unmask the latent ATPase activity of isolated spinach CF_1 [7,29]. Activation is followed by a second cleavage between spinach Lys-204

Organism	1	2	3	4	5	6	7	8	9	10
1 *Synechocystis* 6803	100	73	73	58	55	39	40	38	37	35
2 *Synechococcus* 6301	72	100	79	57	59	37	37	37	36	35
3 *Anabaena* 7120	73	79	100	61	58	38	38	40	36	33
4 c*Spinacia oleracea*	56	55	59	100	61	38	37	37	40	33
5 c*Ch. reinhardtii*	54	58	56	61	100	38	40	39	36	34
6 *E. coli*	43	41	42	43	43	100	43	44	41	35
7 *Rp. blastica*	44	41	42	42	45	43	100	61	41	42
8 *Rs. rubrum*	40	40	42	40	42	42	59	100	40	39
9 PS3	42	41	40	46	41	42	42	42	100	37
10 m*Bos taurus*	40	41	38	39	41	36	45	42	39	100

Fig.3. Binary comparison matrix of γ-subunits. Horizontal reading gives the percentage of identical amino acids of two γ-subunits related to the length of the protein of the vertically listed organisms. Vertical reading shows the corresponding numbers related to the horizontally listed sources of the ATPases.

and Cys-205 which release a cysteine-containing peptide (S2 peptide: -CVDAAEDELFR-, [7,29]). This cysteine, which is not present in the *Synechocystis* sequence, forms a disulfide bridge with the neighboring Cys-199 in oxidized chloroplast ATPase. Upon reduction in illuminated chloroplasts by thioredoxin or DTT, the ATPase alters its regulatory properties ('thiol modulation') thus leading to a metastable active ATPase [5,30]. Similarly isolated spinach CF_1 may be activated by long-time incubation with a high concentration (50 mM) of DTT [31]. Preliminary data with the isolated F_1 from *Synechocystis* 6803 indice that this latent enzyme is activated by trypsin treatment (as other cyanobacterial ATPases, [33,34]), but not by reduction with dithiothreitol (Schumann, J., unpublished data).

Hence, the region between 195–233 seems to include an important regulatory function within the CF_1 complex which allows the enzyme of chloroplasts and cyanobacteria to switch between latent and active states. It is prompting to assume that introduction of this region during evolution has rendered a well-regulated ATPase in photosynthetic organisms which is adapted to the diurnal changes of photosynthetic energy flux.

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