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# The $\gamma$ -subunit of ATP synthase from spinach chloroplasts

Primary structure deduced from the cloned cDNA sequence

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cDNA clones encoding the y-subunit of chloroplast ATP synthase were isolated from a spinach library using synthetic oligonucleotide probes. The predicted amino acid sequence indicated that the mature chloroplast y-subunit consists of 323 amino acid residues and is highly homologous (55% identical residues) with the sequence of the cyanobacterial subunit. The positions of the four cysteine residues were identified. The carboxyl-terminal region of the choloroplast y-subunit is highly homologous with those of the y-subunits from six other sources (bacteria and mitochondria) sequenced thus far.

H<sup>+</sup>-ATPase; F<sub>1</sub>; y-Subunit; Cyanobacteria; cDNA; (Spinach chloroplast)

# 1. INTRODUCTION

ATP synthase  $(CF_0F_1)$  of chloroplast thylakoids catalyzes the light-driven synthesis of ATP from ADP and P<sub>i</sub> [1,2]. The catalytic portion, CF<sub>1</sub>, consists of five different subunits  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$  [3]. Isolated  $CF_1$  is a latent ATPase that can be activated by a variety of methods [4], whereas the  $F_1$ of mitochondria or bacteria already has ATPase activity without such treatments. The  $\gamma$ -subunit of  $CF_1$  is believed to be important in the regulation of ATPase activity and the flow of protons through the proton pathway ( $CF_0$ ) of the enzyme [1,4]. Conformational changes of the  $\gamma$ -subunit, which have been studied by examining alterations in reactivity to sulfhydryl reagents and sensitivity to trypsin [5-8], may be closely related to the regulatory roles of this subunit.

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no.Y00758

The amino acid sequence of the E. coli  $\gamma$ -subunit [9,10] is known from the DNA sequence, and studies with mutants suggested that both the amino- and carboxyl-terminal regions of this subunit are required for assembly of  $F_1$  and its catalytic function [11,12]. The primary sequences of the  $\gamma$ -subunits from Rhodospirillum rubrum [13], Rhodopseudomonas blastica [14], thermophilic bacterium PS3 [15], Synechococcus sp.6301 [16] and bovine mitochondria [17] have also been determined. Here, we cloned and sequenced cDNA coding for the  $\gamma$ -subunit of spinach chloroplasts, since determination of its primary sequence is essential for understanding the regulation of  $CF_1$  and since it was of interest to compare its sequence with those of  $\gamma$ -subunits from other sources. From the predicted amino acid sequence, we identified conserved residues and discuss the significance of the regions containing cysteine residues, which have been studied extensively in relation to conformational change of CF1 [8].

## 2. MATERIALS AND METHODS

Purified CF1 from spinach was subjected to polyacrylamide

Published by Elsevier Science Publishers B.V. (Biomedical Division) 00145793/88/\$3.50 © 1988 Federation of European Biochemical Societies gel electrophoresis in the presence of SDS [18], and the  $\gamma$ subunit was recovered electrophoretically from the gel matrix. The amino-terminal sequence of the  $\gamma$ -subunit from the amino terminus to residue 20 (ANLRELRDRIGSVKNTQKIT) was determined with a gas-phase sequencer (Applied Biosystems). A 20-base oligonucleotide probe [5'-GTIAA(A/G)AA(T/C)ACI-CA(A/G)AA(A/G)AT-3'] (probe N) corresponding to the amino-terminal region between the Val-13 and Ile-19 residues and the 21-base oligonucleotide [5'-(T/C)TC(A/G)TC(T/C)T-CIGCIGC(A/G)TCIAC-3'] (probe S2) corresponding to tryptic peptide S2 [16] were synthesized on an Applied Biosystems DNA synthesizer.

Spinach (*Spinacia oleracea* var. Nobel) seeds were obtained from a local store and grown under conditions of high humidity. After 5–6 days in absolute darkness, the plants were placed under daylight and cotyledons were collected 10 h later. Total RNA was isolated from the tissue homogenate by phenol extraction,  $poly(A)^+$  RNA being isolated by oligo(dT)-cellulose column chromatography [19].

Single-stranded cDNA was synthesized by oligo(dT) priming from mixtures with poly(A)<sup>+</sup> RNA as templates, and doublestranded cDNA by using E. coli DNA polymerase I, RNase H and DNA ligase [20]. Flush ends were generated with T<sub>4</sub> DNA polymerase, and the double-stranded DNA was treated with EcoRI methylase. After ligation with EcoRI linker, the resulting DNA was digested with EcoRI and passed through a Sephadex G-25 column. Flush ended DNA was also directly ligated with EcoRI adapter (Pharmacia). Both DNA preparations were ligated with pUC18 and recombinant plasmids were introduced into E. coli C600 [21]. About 10<sup>4</sup> colonies of both DNA preparations were screened by colony hybridization with probes N and S2 labeled at the 5'-end with <sup>32</sup>P. Nucleotide sequences were determined by the dideoxynucleotide chaintermination method [22]. Other reagents used were as described in [12] or of the highest grade commercially available.

#### 3. RESULTS

We have identified two clones carrying parts of the cDNA for the CF<sub>1</sub>  $\gamma$ -subunit: pSG302 and pSG402 were identified in the cDNA library constructed using *Eco*RI linker and adapter, respectively (fig.1A). The recombinant plasmid pSG302 (carrying 750 bp cDNA) hybridized with both probe N and S2, while pSG402 (carrying 700 bp cDNA) hybridized with only probe S2. Both strands of the inserts of the two plasmids were sequenced and the cDNA coding for the  $\gamma$ -subunit was obtained (fig.1B). pSG402 had an internal EcoRI site, while pSG302 carried a sequence upstream of this EcoRI site, suggesting that methylation with EcoRI methylase was not sufficient for constructing the latter plasmid. The open reading frame was 987 bp long and encoded 329 amino acid residues. This sequence contained the amino-terminal sequence of the mature protein determined chemically (residues Ala-1 to Thr-20), and part of the transit sequence (residues -1 to -6). Thus, the mature protein consisted of 323 amino acid residues of molecular mass 35.7 kDa. which is in good agreement with the value of 35 kDa determined from the mobility of the protein on polyacrylamide gel electrophoresis in the presence of SDS [26]. We identified sequences having a similar amino acid composition to those of the four tryptic peptides (S1–S4) [23] (fig.1B). The amino acid sequence of tryptic peptide S2 [16] was identical with that of the residues between 205 and 215. The overall amino acid composition of the mature  $\gamma$ -subunit was essentially the same as that determined chemically [23].

### 4. DISCUSSION

It is of interest to compare the amino acid sequence of the  $\gamma$ -subunit of spinach chloroplasts with those of six other sources that have been determined so far [9,10,13–17]. The chloroplast sequence had the highest homology (55%) with that of the  $\gamma$ -subunit from the cyanobacterium *Synechococcus* sp., and lower homology with those of the subunits from *E. coli* (33%) and bovine heart (27%), supporting the close evolutionary relationship between chloroplasts and cyanobacteria. When all the  $\gamma$ -subunits thus far sequenced are aligned to obtain maximal homology, only 33 residues are identical. The carboxyl-

Fig.1. Nucleotide and deduced amino acid sequence of the  $\gamma$ -subunit of spinach chloroplast ATP synthase (CF<sub>0</sub>F<sub>1</sub>). (A) Partial restriction endonuclease map of the two cDNA clones pSG302 and pSG402. The reading frame for the  $\gamma$ -subunit is shown by an open box. Both strands of the cDNA clones were sequenced. Sites of restriction endonucleases: Ha, *HaeIII*; H, *HindIII*; S, *SalI*; E, *Eco*RI; B, *BglII*. (B) Nucleotide sequence and deduced amino acid residues of the cDNA coding for the  $\gamma$ -subunit. The amino-terminal sequence of the  $\gamma$ -subunit obtained chemically (residues Ala-1 to Thr-20) is underlined. Regions corresponding to the four tryptic fragments (S1-S4) [23] are also shown. Numbers in parentheses indicate amino acid residues from the amino terminus of the mature  $\gamma$ -subunit.



1090 1100 1110 1120 1130 1140 GTACGTTTTTGTGATGGTTTGAAATGGACAGGGGGATATATCAAGTGCAGAGAGATTAATT

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FEBS LETTERS

Spinach chioropiast      AkleELEDR IGSVKHTAKI TEAMKLVMAA KVRAAGAUV NGRPFSETLV        Svnechococus 6301      MANKAIRDR ISSVKHTKKI TEAMKLVMAA KVRAAGAUV STRPFADRLA        Schlastica      MACAKEIRSK IASVKHTKKI TKAMEMVAAS KNRKAGAUA SARVARAEALSTRPFADRLA        Bovine sitochondrion      MASLKDLKNR ISSVKHTKKI TKAMEMVAAS KNRKAGAUA AARPYAERKA        PS3      MASLKDLKNR ISSVKHTKKI TKAMEMVAAS KLRKAGDSAE AARPYAERKA        Spinach chioropiast      Spinach chioropiast        Svnechococus 6301      EVLYMMEGU GTEDVOVLT KLRVKVAL NVV-CORCU GGGVNSVH        Svnechococus 6301      KVIGHLAGARAEKK LGUDVFLT KLRVKVAL LVV-SORGL GGGVNSVH        Svnechococus 6301      KVIGHLAGACHUVLT SIGHKGMUTTI K-REPUVDFL GGGLNNLFR        Svnechococus 6301      KVIGHLAGACHUVLT SIGHKGMUTTI K-REPUVDFV V-SORGL GGGNSIN        Svnechococus 6301      KAESRIAELK KLGUDVTII SIGHKGMUTTI K-REPUVD V-POCTULF        Svnechococus 6301      KAESRIAELK KLGUDVTII SIGHKGMUTTI R-REPUVD V-VOLSUKROV        Svnechococus 6301      KAESRIAELK KLGUDVTII SIGHKGMUTTI R-REPUVD V-VOLSUKROV        Svnechococus 6301      KAESRIAELS AGKUDVKFU USRPFSKI LDUNKROV V-VOLSUKROV        Svnechococus 6301      KKAEGALADVKYFU USRPFKKTGV LVI-TSDRCL GGGNSNVRU        Svnechococus 6301      KKAEGALADVKYFU USRPFKKTGV LVI-TSDRCL GGGNSNVRU        Svnechococus 6301      KKAEGALADVKYFU USRPFKLTGV SVSEV		1			_ 49
Synechococcus6301MANLKAIRDN IKSVRNTRKITEAMRLYMAA KVRRAGUVL STRPFADRIA§: oliasticaMANLKAIRDN IKSVRNTRKITKAMENYMAA KVRRAGUVL STRPFADRIA§: vubrusMASLKDLERSTSVKSTRKITKAMENYMAA KURRAGUVL STRPFADRIABovine stiochondrionATLKDITRLKSIKNIGKITKSMKNYMAA KVRRAERLK PARYPS3Spinach chloroplastSoSoSvechococcus6301VUACLURK LGEVRNPLLREVKNYLKLVV-TCRFCUSublasticaAVIASLASC GACAPRLIAGNGRDUHL-LVV-TCRFCUCGCUNNIKSoSoSoSoSoSoSublasticaAVIASLASC GACAPRLIAGNGRDUHL-LVV-TCRFCUSolasticaAVIASLASC GACAPRLIAGNGRDUHL-LVV-TCRFCUCGCUNNIKSoSuchostondrionFGVGSLALVE KADI-KTP EDKKNKI LVV-TCRFCUCGCUNNIKSoSuinach chloroplastKKSRKARLEX AGLOVKYI VSRPVKKGV LVI-SDRCL CGCHNSIKCGCUNNIKSynach chloroplastKAESRIAELK KLGVDYTIS IGKKGNTYFI R-REPIPVDR Y-PCGLAITSASovine sitochondrionCKSSLALVE KADI-KTY-LIGKKGRSFN S-VGCWAQ Y-TCHCDNFSovine sitochondrionARKAGAIAD VFSLFYSEVNEVENUTYFI KSWKARDY R VUSSNKLIGIADUSSKALUSynechococcus 6301LARKARLEX AGLOVKYI VGKKAGUYG R VENEVYTK YLLEVGKNPTIIDTRLPDQFSSovine sitochondrionSSLIGPKW HLARKEXKIE GUNKKSUN KENKSNVI VUSSNKLIGIADUSSKALUSpinach chloroplastAREAGAIAD VFSLFYSEVNEVENUTYFI KSKSVITSAESNALUSpinach chloroplastAREAGAIAD VFSLFYSEVNEVENUTYFI SUSSWAPTI SULPLSpinach chloroplast	Spinach chloroplast	ANLRELRDR IGSVKNTQ <sub>i</sub> k <mark>i</mark>	TEAMKLVAAA	KVRRAQEAVV	NGRPFSETLV
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R. F. Cubrus      MASE KRUKSK      ITSVKSTURI      ISJ MARMINAS      KUKARADEREL AND VILATE        Svince mitochondrion      ASLRDIKTR      INATKATSQL      TKSINKIANI      ISJ MARKANAA      KVARAEREL K      PADV        Svince mitochondrion      SULVINHERL      GENVENTER      TKSINKIANI      TKSINKIANI      KVARAEREL K      PADV        Svince mitochondrion      SVICHALAGA      VVARAEREL K      PADV      SVICHALAGA      VVARAEREL K      PADV        Svince mitochondrion      SVICHALAGA      VVARAEREL K      GGTKNDWHL      VVALTANECL      GGGFKNSVIR        Svince mitochondrion      SVICHALAGA      VVARAEREL K      GGTKNDWHL      VVALTANECL      GGGFKNSVIR        Svince mitochondrion      SVICHALAGA      VVANALAA     RASHPHL VSRPVKKTGV      LVV-TSDECL      GGGFKNSVIR        Svince mitochondrion      SVICHALAGA      VVANALAA     RASHPHL VSRPVKKTGV      LVI-TSDECL      GGGFKNSVIR        Svince chococcus      G301      KAESRIAELS      AGGYNKKVIL      VV-TSCRCL (GGFKNSVIR        Svinchococcus      G301      KKESRIAELS      AGGYNKKVIL      VV-TSCRCL (GGFKNSVIR        Svinchococcus      G301 <t< td=""><td><u>R</u>.<u>blastica</u></td><td>MPSLKDLKNR IGSVKNTRKI</td><td></td><td>KLKKAUDSAE</td><td>AARPYAERMG</td></t<>	<u>R</u> . <u>blastica</u>	MPSLKDLKNR IGSVKNTRKI		KLKKAUDSAE	AARPYAERMG
Bovine mitochondrion    ATLKDJTKK LKSIKHUKLI TKATKKTGU    Bovine mitochondrion    GGCKUNSWIN    CGAINSVING    CGAIN	<u>R</u> . <u>rubrum</u>	MASLKDLRSR ITSVKSTQKI	TIS AIMIK MIVIA A S	RLKRAUDTAE	AARPYTURME
Spinach chloroplastSpinach chloro	Bovine mitochondrion PS3	ATLKDITRR LKSIKNIQKI ASIRDIKTR INATKKTSOI	TIK SMIK MIVIA A A TIK AMIE MIVIL T S	KYAKAEKELK Klnraekr-e	
Spinach chloroplast    Evynencococcus 6301    Evynencococcus 6301    Evynencococcus 6301    Evynencococcus 6301    Evynencococcus 6301    Evynencoccus 6301    Evynencocccus 6301    Evynencoccus 6301	155	50			
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PS3    EVVANVALAA   RASHPML    VSRPVKKTGV    LVI-TSD <u>RGL</u> AGGAVNSNVLR      Spinach    chioroplast	Bovine mitochondrion	-GVGSLALYE KADI-KTP	EDKKKHL	IIGVSSDRGL	C G A I H S S V A K
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Synechococcus6301RAEGRARELSAGGLDVKFVIVGGRVAGVPGR-REQPIEATY-SGLEQIPTBolasticaKLIAEMKTUTDKCVGLDAHIGSKGVSFNS-VGGNVVAQY-GKGDDSKAIGRrubrusmitochondrionMKSEAAHLAAGKEVVILLIGSKGVSFNS-VGGNVVAQY-GKGDDSKAIGPS3mitochondrionMKSEAAHLAAGKEVVILLIGKKGRDGLKREPPKQIIGGIADQSSKAIGSpinachchioropiastAKEAQAIADDVFSLFVSEEVDKVEMLYTKFVSLVKSDPVIHTLLPDPGSpinachchioropiastAKEAQAIADDVFSLFVSEEVDKVEMLYTKFVSLVKSDPVIHTLLPDPGBotasticaKEAQAIADDVFSLFVSEEVDKVEMLYTKFVSLVKSDPVIHTLLPDPGRtubrusSvandgiareVLAAFEAGEADVVTTKFSUSASAPTAQUIPAK-BovinemitochondrionFSDADRFSRLILDMFQAGEFDVCLVYRFFQAISQVTRQUIPAK-FADIKEIARKTVGRKAGVSFFFSDIACHFSDADRFSRLILDMFQAGEFDVCLVYRFFQAISQVTRQUIPAK-SpinachchioropiastSynechococcus6301	Spinach chloroplast	KAESRIAELK KLGVDYTIIS	IGKKGNTYFI	R - R P E I P V D R	Y-FDGTNLPT
E. coliKLLAEMKTVT DKGVQCDLAMIGSKGVSFFN S-VGGNVVAQV-TCMGDMPSS. blasticaLARQANALCU AQGKTVKLLY (GKKGREQLK REPPKQ1 GG IADQSSKAIGAARGXNULLC  GKKGRDGLK REPPKQ1 GG IADQSSKAIGBovine #itochondrionQMKSEAANLA AAGKEVKILG VGDKIRDGLK REPPKQ1 GG IADQSSKAIGSyinach chloroplastAKEAQAIADD VFSLFVSEEVDKVEMUYTKF VSLVKSDPVI HTLLPLSPKGS. coliAKEAQAIADD VFSLFVSEEVDKVEMUYTKF VSLVKSDPVI HTLLPLSPKGB. coliAKEAQAIADD VFSLFVSEEVDKVEMUYTKF VSLVKSDPVI HTLLPLSPKGS. coliAKEAQAIADD VFSLFVSEEVDKVEMUYTKF VSLVKSDPVI HTLLPLSPKGB. coliSELIGPVKV MLQAVDEGRLDKVIVSKFF INTMSQVPTI QUIPFAVFTBovine #itochondrionFGDASTRI LLDHFQAGEFDVCTLVYNKF QSJISQVTA QUIPFAVFTFGDASTRI LDHFQAGEFDUCTLVYNKF SSISQVTR QUIPFAVFTBovine #itochondrionFGDASTRI LDHFQAGEFDUCTLVYNKF RSVISCOVPS3Spinach chloroplastEICDINGKCV DAAEDELFRL TTKEGKLTVE RDHIKTETPA FSPILE-FEQSpinach chloroplastSPINACH Chloroplast	<u>Synechococcus</u> 6301	RAEQRARELS AQGLDYKFVI	VGRKAGQYFQ	R-REQPIEAT	Y-SGLEQIPT
R. blastica Bovine #itochondrion PS3LARGRANELV AGGKTVKILL VICKGREGLK ROVASAFVGH VDLSDVRRLG GKKGRDGLK REFPKallG IADDSSKAIG GKKGRDGLK REFPKallG IADDSSKAIG GKKGRDGLK REFPKallG IADDSSKAIG GKKGRDGLK REFPKallG IADDSSKAIG GKKGRDGLK REFPKallG IADDSSKAIG GKKGRDGLK REFPKallG IADDSSKAIG GKKGRDGLK REFPKallG IADDSSKAIG GKKGRSFF- RKRMMPVIL DITKLPDGPG Spinach chloroplast Spinach chloroplast SciellDall PKV SiellDall PKVV SiellDall PKVVNKARQ ASI TORLY SiellDall PKVNKARQ ASI TORLY SiellDall PKVNKARQ ASI TORLY SiellDall PKVNKARQ ASI	<u>E.coli</u>	KLLAEMKTWT DKGVQCDLAM	1 G S K G V S F F N	S - V G G N V V A Q	V - TGMGDNPS
R. rubrumATRLVKELEAGGKTVKLLCIGKKGNGLKREFFRUITGGIAUGSSKATGBovinemitochondrionGMKSEANLAAAGKTVKLLCIGKKGNGLKREFFRUITGGIAUGSSKATGSpinachchloroplastGMKSEANLAAAGKTVKLLCIGRVGLSFFRKRMMPVLLDITRLPDQPS147196SpinachchloroplastAEANDAAECANDLADELUSTFVSEEVDKVEMLYTKFVSUKSDPVIHTLLPDQPS8. rubrumFSDADRFSRLLUMF4AGEFDVVTIFVNRFQSVISQVPTAQUIPAK-8. rubrumFSDADRFSRLLUMF4AGEFDVCTLVYNRFQSAIQUVTRQUIPAK-8. rubrumFSDADRFSRLLUMF4AGEFDVCTLVYNRFQSAIQUVTRQUIPAK-953FDADKSRLLUMF4AGEFDVCTLVYNRFQSAIQUVTRQUIPAK-953Spinachchloroplast199205245910Scoli199205245245920SpinachGS01ASSDDELFRLTTKEGKLTVERDMIKTETPA8. blasticaFubrumTVA	R.blastica	LARQRANELV AQGKTVKILT	VGKKGREQLK	RDWASAFVGH	VDLSDVRRLG
Bovine mitochondrion PS3OKKEANALA AAGKEVKIIG VIGRVGLSFF- RKRNMPVIL DITRLPDQFS147196Spinach chloroplast <u>Succhococcus</u> 6301AKEAQAIADD VFSLFVSEEV AQEANDIADE LLSLFLSGTV DRVELVYKF VSLVXSDPVI HTLLPLDPQG DRVELVYKF USLVSKSDPVI HTLLPLDPQG DRVELVYKFF USLVSKPVN QTLLPLDPQG DRVELVYKFF QSAISQVPTI QQVIPKF FSDADRFSRL ILDMFGAGEF DVCTLVYNKF GSAISQVVTR QQIIPFAVPT FSDADRFSRL ILDMFGAGEF DVCTLVYNKF QSAISQVVTR QQIIPFAVPT FSDADRFSRL ILDNSQV-EF DEGSIIFNRF RSVIS FADIKEIARK TVGLFADGTF DELVMVYNHV VSAIQQEVTE RKLLPL FADIKEIARK TVGLFADGTF DELVMVYNHV VSAIQQEVTE RKLLPL PASDDDDL	<u>R. rubrum</u>	ATRTLVRELE AQGKTVKLLC	IGKKGRDGLK	REFPKQIIGG	IADUSSKAIG
PS3LVVUTIUKKH ASPDEVAILVIMAVGLSFFKKKNAPVILDIKLPUUPSSpinach chloroplast147196S.blasticaAKEAQAIADDVFSLFVSEEVDKVEHLYTKFVSLVKSDPVIHTLLPLSFKGB.blasticaYSAQGIAREVLAFFAGEADVVTIFVNFFQSLISQVTAQQUIP-AK-B.vine mitochondrionFSDADRFSRLILDMFQAGEFDVCTLVNRFQSLISQVTAQQUIP-AK-FSDInach chloroplastSynechococcus6301LASSDELFRLTTKEGKLTVERDMIKTETPASpinach chloroplastSynechococcus6301LASSDDELFRLTTKEGKLTVERDMIKTETPAFSPILE-FEQSpinach chloroplastSynechococcus6301LASSDDELFRLTTKEGKLTVERDMIKTETPAFSPILE-FEQSpinach chloroplastSynechococcus6301	Bovine mitochondrion	QMKSEAANLA AAGKEVKIIG	VIGIDIKTRSTLH	RTHSDQFLVT	FKEVGRKPPT
147196Spinach chloroplast Synechococcus 6301 E.coliAREAQAIADD VFSLFVSEEV AREAQIADD UFSLFVSEEV DKVEHLYTKF VSLVKSDPVI HTLLPLSPKG DRVELVYTKF USLVASPPV TLLPLDPGG DKLYIVSNKF INTMSQVPTI SQLLPL YSNAQGIARE VLAAFEAGEA DVCTLYVNKF QSVISQVPTA QQVIPAK- FSDADFSRL ILDMFGAGEF DVCTLYVNKF QSVISQVPTA QQVIP AQUIFFANPA FSDADFSRL ILDMFGAGEF DVCTLYVNKF QSVISQVPTA QQVIP AQUIFFANPA FSDADFSRL ILDMFGAGEF DVCTLYVNKF QSVISQVPTA QQVIF RKLLPL FSDADFSRL ILDMFGAGEF DVCTLYVNK ARQAAA SDADFSRL ILDMFGAGEF DVCTLYVNK ARQAAA SDADFSRL ILDMFGAGEF DVCTLYVNK ARQAAA SDADFSRL ILDMFGAGEF DVCTLYVNK ARQAAA SDADFSRL ILDMFGAGEF DVCTLYVNKAAAA SDADFSRL ILDMFGAGEF DVCTLYVNKAAAAA SDADFSRL ILDMFGAGEF DVCTLYVNKAAAAA SDAAAA SDAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	PS3	LVYUTIUKRH ASPDEYATIV	IGKVGLSFF-	-KKKNMPVIL	UTIKLPUUPS
Spinach chloroplast Synechococcus 6301AKEAQAIADD VFSLFVSEEV QRVELVTKF LSLVASNPVV QTLLPLSPKQ QRVELVTKF DRVELVTKF LSLVASNPVV QTLLPLDPQQ QRVELVTKF LSLVASNPVV QTLLPLDPQQ LSELIGPVKV HQAVDEGRL DVCTLVVNRF QSVISQVTA QQVIPAK- FSDADRFSRL ILDMFQAGEF DVCTLVVNRF QSVISQVTA QQVIPAK- FSDADRFSRL ILDMFQAGEF DVCTLVVNRF QSVISQVTA QQVIPAK- FSDADRFSRL ILDMFQAGEF DVCTLVVNRF QSVISQVTR QQIIPFAVPT FGDASVIALE LUNSGV-EF DECSIFRKF RSVIS FADIKEIARK TVGLFADGTF DELYMYVNHV VSAIQQEVTE RKLLPL 199 205 Spinach chloroplast Synechococcus 6301 F.coli R. rubrum PS3245 246Spinach chloroplast Synechococcus 6301 C.coli R. rubrum Synach chloroplast Synach chlorop		147	_		196
Synechococcus6301 E.coliAQEANDIADELLSLFLSGTVDikvelvytkfLSLVASNPVQTLLPLDPQQ SQLPLE.blastica R.rubrumYSNAQGIAREVLAFEAGAGEFDVVTIFYNKFQSVISQVPTAQQLIPFAVPTBovine Titochondrion PS3FSDADRFSRLILDNSGVEFDEGSIIFNKFRSVISQVPTAQQLIPFAVPTSpinachchloroplast Svnechococcus6301 E.coli R.blastica R.rubrum245245Spinachchloroplast Svnechococcus6301 E.coli R.blastica R.blastica245Spinachchloroplast Svnechococcus700 KEI F.S.DADRIFNETTKEGKLTVE R.Coli R.blastica R.blastica R.blasticaTV-A F.F.S.DADRIFNE TV-A	Spinach chloroplast	AKEAQAIADD VFSLFVSEEV	DKVEMLYTKF	VSLVKSDPVI	HTLLPLSPKG
E. coli R. blastica Novine mitochondrion PS3LSELIGPVKV MLQAYDEGRL DKLYIVSNKF INTMSQVPTA QQVIPAK- YSNAQGIARE VLAAFEAGEA DVVTIFYNRF QSVISQVPTA QQVIPAK- PSDADFSRL ILDMFQAGEF DELYMYYNRF QSAISQVPTA QQVIPAK- PSDADFSRL ILDMFQAGEF DELYMYYNRF VSAIQQEVTE RKLLPLSpinach chloroplast Synechococcus 6301 E. coli R. blastica R. rubrum PS3199205 EICDINGKCV DAAEDELFRL TTKEGKLTVE RDMIKTETPA FSPILE-FEQ TTKGGSFTVE REKLTSEVAP LPRDMI-FEQ L ASDDDDL 	<u>Synechococcus</u> 6301	AQEANDIADE LLSLFLSGTV	DRVELVYTKF	LSLVASNPVV	QTLLPLDPQG
R. blastica R. rubrumYSNAQGIARE SDADRFSRL FSDADRFSRL SDADRFSRL LILDMFQAGEF FSDADRFSRL DVCTLVYNRF SDADRFSRL LILDMSQY-EF DECSIJFNRF DECSIJFNRF DELYMYNNHY VSAIQQEVTE RKLLPLQQUIPFAVPT QAISQVTR QUIPFAVPT FQDASVIALE LLNSGY-EF DECSIJFNRF DELYMYNNHY VSAIQQEVTE RKLLPLSpinach chioroplast Synechococcus 6301 E.coli R. blastica R. blastica R. rubrum205 EICDINGKCV AASDDEJFRL TV-A	<u>E.coli</u>	LSELIGPVKV MLQAYDEGRL	DKLYIVSNKF	INTMSQVPTI	SQLLPL
R. rubrumFSDADRFSRLILDMFQAGEFDVCTLVYNRFQSAISQVTRQUIIPFAVPTBovinemitochondrionFGDASVIALELLNSGY-EFDEGSIIFNRFRSVIS	<u>R.blastica</u>	YSNAQGIARE VLAAFEAGEA	DVVTIFYNRF	QSVISQVPTA	QQVIPAK-
Bovine mitochondrionFGDASVIALELUNSGY-EFDEGSIIFNEPKSVISPS3FADIKEIARKTVGLFADGTFDELYMYYNHYVSAIQQEVTERKLLPLSpinachchioropiast199205245Svnechococcus6301LASSDDEIFRLTTKEGKLTVERDMIKTETPAF. blasticaLASDDDL	R. <u>rubru</u>	FSDADRFSRL ILDMFQAGEF	DVCTLVYNRF	QSAISQVVTR	QQIIPFAVPT
PS3    FADIKETARK IVGLPADGIF WELPHVYNHY VSATUUEVIE KKLLPL      199    205      Spinach chloroplast    199      Svnechococcus 6301   L      K. coli   L      R. cubrum   L      Bovine mitochondrion       PS3    TVA      Spinach chloroplast	Bovine mitochondrion	FGDASVIALE LLNSGYEF	DEGSTIFNEF	KSVIS	
199205245Spinach chloroplastEICDINGKCVDAAEDELFRLTTKEGKLTVERDMIKTETPASynechococcus6301LASSDDEIFRLTTKEGKSTVEREKLTSEVAPLPRDMI-FEQR. blasticaFASDDDDL	P 5 3	FAUIKEIAKK IVGLFADGIF	DELIMITANT	VSAIUUEVIE	KKLLPL
Spinach chloroplast Synechococcus 6301EICDINGKCV DAAEDELFRL TTKEGKLTVE ROMIKTETPA FSPILE-FEQ ASSDDELFRL TTREGSFTVE REKLTSEVAP LPRDMI-FEQ PASDDDLKHKSVD YLYQP PALYD-YEP FAAE-TAG NDNDRTAGPKAIYE-YEP NALYD-YEP TV-AYKTEE KPIFSLDTIS SAESMSIYD DPAQILLOALL PLYLNSQILR ALQESLASEL AARMTAMNSA DPAQILLSALL PLYLSNQLLR ALQESLASEL AARMTAMNSA DPAQILLSALL PLYLSNQLLR ALQESLASEL AARMTAMNSA SDNANALYGQ 295246 295 296 Spinach chloroplast Synechococcus 6301 E.coli R.rubrum PS3246 295 296 296 296 295 296 296 296 296 296 296 296 296 296 296 296 296 297 296 296 296 296 296 296 296 297 296 296 296 296 296 296 297 296 296 296 296 297 296 296 296 296 296 297 296 297 296 296 296 296 297 296 297 296 296 297 296 296 297 296 297 296 296 297 296 297 296 297 296 296 297 296 297 296 297 296 297 296 297 296 297 296 297 296 297 296 297 296 297 296 297 296 297 298 298 298 298 299 2090 2000 <b< td=""><td><b>.</b></td><td>199 205</td><td></td><td></td><td>245</td></b<>	<b>.</b>	199 205			245
Synechococcus6301PASSDDDL-FKLTIKGGSFTVEKEKLTSEVAPLPKDMI-FEQE. coliPASDDDLPKHKSWDYLYEPBovinemitochondrion	Spinach chloroplast	EICDINGKCV DAAEDELFRL	TTKEGKLTVE	RDMIKTETPA	FSPILE-FEQ
L. coli    Find true      R. blastica    TV-A      Bovine mitochondrion    TV-A      PS3    Spinach chloroplast      Synechococcus 6301    DPAQILDALL PLYLNSQILR ALQESLASEL AARMTAMISNA TONANELKKT      Bovine mitochondrion    DPAQILDALL PLYLNSQILR ALQESLASEL AARMTAMISNA TONANLKKT      Spinach chloroplast    DPAQILDALL PLYLNSQILR ALQESLASEL AARMTAMISNA TONASLKKT      Synechococcus 6301    DPAQILDALL PLYLNSQULR ALQESLASEL AARMTAMISNA TONASDINANALVGQ      R. blastica    SEAILADLL PRGVATQIFT ALLENAASEQ GARMSAMDNA TRNAGDMINK      Bovine mitochondrion    IDADVLRNYQ EYSLANIIYY SLKESTTSEQ SARMTAMONA SKNASEMIDK      Spinach chloroplast    SQEEILDVLL PQYAESLIYG ALLDAKASEH AARMTAMISNA TONANELIRT      296    LSINYNRARQ AAKTIGELLEY VAGANAQU      Spinach chloroplast    SQEEILDVLL PQYAESLIYG ALLDAKASEH AARMTAMINA TRNAGDMINK      Solastica    LSINYNRARQ AAKTIGELLEY VAGANAQU      R. blastica    LISINYNRARQ AAKTIGELLEY VAGAAAQU      R. blastica    LISIYNRKRQ AALTKELIEI ISGAEAL      R. blastica    LISIYNRKRQ AQU      R. blastica    LISIYNRKRQ AQU      R. blastica    LISIYNRKRQ AQU      R. blastica    LISIYNRKRQ AQU      R. blastica    LISIYNRKRQ AALTKELIEI	Synechococcus 6301	L ASSUDEIFKL	TTRGGSFTVE	REKLTSEVAP	EPROMI-FEQ
R. <u>rubrum</u> TV-A	$\underline{\mathbf{L}} \cdot \underline{\mathbf{COII}}$	F FAF T		KHK5WD	YLYEP
R. Lubrum    Intervent	R. UIASTICA Prubrum	TV-A		NONDETACE.	- NALTU-TEP
PS3    246    295      Spinach chloroplast    DPAQILDALL PLYLNSQILR ALQESLASEL AARMTAMSNA    TDNANELKKT      Synechococcus 6301    DPAQILLSALL PLYLNSQILR ALQESLASEL AARMTAMNSA    SDNANALVQQ      E.coli    DPAQILLSALL PLYLSNQLLR ALQESLASEL AARMTAMNSA    SDNANALVQQ      R.mubrum    DPAQILLSALL PLYLSNQLLR ALQESLASEQ AARMVAMKAA    SDNANALVQQ      Bovine mitochondrion    SEEAILADLL PRGVATQIFT ALLENAASEQ GARMTAMDNA    SDNANALVQQ      Spinach chloroplast    SEEEILADLL PKNVAIQVFR GMLESFASEQ GARMTAMDNA    TRNAGDMIKK      Spinach chloroplast    SQEEILDVLL PQVAESLIVG ALLDAKASEH    SARMTAMDNA      Spinach chloroplast    SQEEILDVLL PQVAESLIVG ALLDAKASEH    SARMTAMDNA      Spinach chloroplast    SQEEILDVLL PQVAESLIVG ALLDAKASEH    SARMTAMDNA      Spinach chloroplast    SQE    SQE    SQ22      Spinach chloroplast    LSINVNRARQ AAITQELLEI VAGANALV    SQARMTAMDNA      Spinach chloroplast    LUVNKARQ AAITQELLEI VSGAAAV    SQ22      Spinach chloroplast    LSINVNRARQ AAITQELEI SGAAAV    LIVYNKARQ AAITQELEI SGAAAV      LUVYNKARQ AAITQELEI VSGAAAV    LIVYNKARQ AAITGELIEI SGAEAL    LSLTYNRTRQ AQITKELIEI ISGAEAL      LSLTYNRARQ AAITKELIEI VAGANALQ    LSLTYNRARQ AAITGELIEI VAGANALQ   <	Roving mitochondrion			KDIECINTIC	CAECMCIVDD
246295Spinach chloroplastDPAQILDALL PLVLNSQILR ALQESLASELAARMTAMSNASynechococcus 6301DPAQILLSALL PLVLSNQLLR ALQEAAASELAARMTAMNSAE.coliDPAQILLSALL PLVLSNQLLR ALQEAAASEQAARMVAMKAAR.rubrumDPAQILLALL PRGVATQIFT ALLENAASEQAARMVAMKAABovine mitochondrionSEEILADLL PRGVATQIFT ALLENAASEQGARMSAMDNAPS3SeeILDVLL PGVAESLIVG ALLDAKASEHSKNASEMIDKSpinach chloroplastSQEEILDVLL PQVAESLIVG ALLDAKASEHSKNASEMIDKSpinach chloroplastSQEEILDVLL PQVAESLIVG ALLDAKASEHSKNASEMIDKSpinach chloroplastSQEEILDVLL PQVAESLIVG ALLDAKASEHSKNASEMIDKSpinach chloroplastSVNRARQ AAITQELLEV VAGAAAVSKNASEMIDKLSINVNRARQ AAITQELLEI SGAAAVLTIQVNKARQ AAITQELLEI SGAEALSGAAAVLLVVNKARQ AAITTKELIEI ISGAEALLSLTVNRTRQ AQITKELIEI ISGAEALLSLTVNRTRQ AQITKELIEI ISGAEALPS3LTICTNRTRQ AQITKELIEI ISGAEALLSLTVNRARQ AAITTGELTEI VAGANALQ	PS3		TDLAEN	K0	-RTVYE-FEP
Spinach chloroplastDPAQILDALLPLYLNSQILRALQESLASELAARMTAMSNATDNANELKKTSynechococcus6301DPAQILDALLPLYLSNQLLRALQESLASELAARMTAMNSASDNANALVGQE.coliDPAQILDALLPLYLSNQLLRALQEAAASELAARMTAMNSASDNANALVGQR.blasticaDPKALLDTLLRRYVESQVYQGVVENLASEQAARMVAMKAATDNGGSLIKEBovinemitochondrionSEEAILADLLPRGVATQIFTALLENAASEQGARMSAMDNATRNAGDMINKPS3SEEEILADLLPKNVAIQVFRGMLESFASEQGARMTAMDNASKNASEMIDKSpinachchloroplastSQEEILDVLLPQVAESLIYGALLDAKASEHAARMTAMNNATDNANELIRT296322SpinachchloroplastSQEEILDVLLPQVAESLIYGALLDAKASEHAARMTAMKNATDNANELIRT296SinaticaLSINVNRARQAAITTGELLEVVAGAAAAVTDNANELIRTSUBANELIRT296S22SpinachChloroplastSUBANALVGQSUBANALVGQE.coliLUVNKARQAAITTGELLEVVAGAAAAVTDNANELIRT296S22SUBANALVGQSUBANALVGQSUBANALVGQE.coliLUVNKARQAAITTGELLEVVAGAAAAVLUVNKARQAAITTGELLEVVAGAAAAVLUVNKARQAAITTGELTEIVSGAAAVLUSLTYNRTRQAQITKELLEIISGAAALPS3LTLSYNRARQAAITTGELTEIS0LTLSYNRARQAAITTGELTEIF3SUBANALVQQSUBANALVQQF3SUBANALVQQSUBANALVQQS0SUBANALVQQ <t< td=""><td></td><td>246</td><td></td><td></td><td>295</td></t<>		246			295
Synechococcus    6301      Synechococcus    6301      Coli    DPAQILSALL      R.blastica    DPAQILSALL      R.rubrum    SEEAILADLL      Bovine    Bitochondrion      PS3    SEEEILADLL      PS3    SeeEILDVLL      PS3    SeeEILDVL      PVNARRQ    AAITTGEILEV      PS3    SeeEILDVL      PS3    Se	Spinach chloroplast	DPAGILDALL PLYENSOLLR	ALGESLASEL	AAR MITAMS NA	TONANELKKT
E.coli    DPKALLUDTLL RRYVESQVYQ GVVENLASEQ AARMVAMKAA      R.blastica    SEEAILLADLL PRGVATQIFT ALLENAASEQ GARMSAMDNA      R.rubrum    SEEAILLADLL PRGVATQIFT ALLENAASEQ GARMSAMDNA      Bovine mitochondrion    IDADVLRNYQ EVSLANIIVY SLKESTTSEQ SARMTAMDNA      PS3    SQEEILDVLL PQVAESLIYG ALLDAKASEH      296    322      Spinach chloroplast    LSINVNRARQ AAITQELLEV VAGAEALNG      E.coli    LSINVNRARQ AAITQELLEV VAGAEALNG      E.coli    LQLVYNKARQ AAITQELLEI SGAEAL      R.rubrum    LSINVNRRRQ AQITKELIEI ISGAEAL      Bovine mitochondrion    LTIYNRRRQ AQITKELIEI ISGAEAL      PS3    LTIYNRRRQ AQITKELIEI ISGAEAL	Synechococcus 6301	DPAQILISALL PLYLSNOLLR	ALQEAAASEL	A A R MITA MIN SIA	SDINANALVGO
R. blastica    SEEAILADLL    PRGVATQIFT    ALLENAASEQ    GARMSAMDNA    TRNAGDMINK      R. rubrum    SEEEILADLL    PRVATQIFT    ALLENAASEQ    GARMSAMDNA    TRNAGDMINK      Bovine    mitochondrion    SEEEILADLL    PKNVAIQVFR    GMLESFASEQ    GARMSAMDNA    TRNAGDMINK      PS3    SQEEILDVLL    PQVAESLIVG    ALLDAKASEH    AARMTAMDNA    SKNASEMIDK      296    322      Spinach    chloroplast    SVNARARQ    AAITQELLEV    VAGABANACV <u>Synechococcus</u> 6301    LSINVNRARQ    AAITQELLEV    VAGABANV <u>E. coli</u> LQLVYNKARQ    ASITQELTEI    VSGAAAV <u>Bovine</u> mitochondrion    LTIQYNRSRQ    AAITTKELIEI    ISGAAAV      Bovine    mitochondrion    LTITFNRTRQ    AVITKELIEI    ISGAAAL      PS3    LTLSYNRARQ    AAITTGEITEI    VAGABAAL	E.coli	DPKALLOTLL RRYVESQVYQ	GVVENLASEQ	A A R MIVA MIK A A	TONGGSLIKE
R. rubrum    SEEEILADLL PKNVAIQVFR GMLESFASEQ GARMTAMDNA Bovine mitochondrion    TRNAGDMIKK IDADVLRNYQ EYSLANIIYY SLKESTTSEQ SARMTAMDNA SQEEILDVLL PQVAESLIYG ALLDAKASEH AARMTAMDNA SQEEILDVLL PQVAESLIYG ALLDAKASEH AARMTAMKNA TDNANELIRT      296    322      Spinach chloroplast Synechococcus 6301    LSINVNRARQ AAITQELLEV VAGAEALNG LLVYNKARQ ASITQELTEI VSGAAAV LLVYNKARQ AAITTKELIEI ISGAEAL LLVYNKARQ AAITKELIEI ISGAEAL LSLTYNRTRQ AQITKELIEI ISGAEAL LSLTYNRTRQ AQITKELIEI ISGAEAL LLTLSYNRARQ AAITQEITEI VAGANALQ	R. blastica	SEEAILADLL PRGVATQIFT	ALLENAASEQ	GARMSAMDNA	TRNAGDMINK
Bovine mitochondrion    IDADVLRNYQ EYSLANIIYY SLKESTTSEQ SARMTAMDNA SKNASEMIDK      PS3    SQEEILDVLL PQVAESLIYG ALLDAKASEH AARMTAMKNA TDNANELIRT      296    322      Spinach chloroplast    LSINYNRARQ AAITGELLEY VAGANACV <u>Synechococcus</u> 6301    LTLVYNKARQ AAITGELLEY VAGAEALNG <u>E.coli</u> LQLVYNKARQ AAITGELLEI VSGAAAV <u>R. rubrum</u> LSINYNRSRQ AAITKELIEI ISGAEAL      Bovine mitochondrion    LTLTYNRTRQ AVITKELLEI ISGAEAL      PS3    LTLSYNRARQ AAITGELTEI VAGANALQ	<u>R. rubrum</u>	SEEEILADLL PKNVAIQVFR	GMLESFASEQ	GARMTAMDNA	TRNAGDMIKK
PS3    SQEEILDVLL PQVAESLIYG ALLDAKASEH AARMTAMKNA TDNANELIRT      296    322      Spinach chloroplast    LSINYNRARQ AKITGEILEI VAGANACV <u>Synechococcus</u> 6301    LTLVYNKARQ AAITQELLEV VAGAEALNG <u>E.coli</u> LQLVYNKARQ ASITQELTEI VSGAAAV <u>R.blastica</u> LTIQYNRSRQ AAITKELIEI ISGAEAL <u>R.rubrum</u> LSLTYNRTRQ AVITKELIEI ISGAEAL      Bovine mitochondrion    LTLTSYNRARQ AAITQEITEI VAGANALQ	Bovine mitochondrion	IDADVLIRNYQ EYSLANIIYY	SLKESTTSEQ	SARMTAMD NA	SKNASEMIDK
296    322      Spinach chloroplast    LSINYNRARQ AKITGEILEI VAGANACV      Synechococcus 6301    LTLVYNKARQ AAITGEILEV VAGAEALNG      E.coli    LQLVYNKARQ ASITGEILEI VSGAAAV      R.blastica    LTIQYNRSRQ AAITKELIEI ISGAEAL      R.rum    LSLTYNRTRQ AQITKELIEI ISGAEAL      Bovine mitochondrion    LTLTSYNRARQ AAITGEITEI VAGANAL      PS3    LTLSYNRARQ AAITGEITEI VAGANALQ	P S 3	SQEEILDVLL PQYAESLIYG	ALLDAKASEH	AARMTAMKNA	TOMANELIRT
Spinach    chloroplast    LSINVINRARQ    AKITGEILEI    VAGAGNACV      Synechococcus    6301    LTLVYNKARQ    AAITGELLEV    VAGAEALNG      E.coli    LQLVYNKARQ    ASITGELTEI    VSGAAAV      E.coli    LQLVYNKARQ    ASITGELTEI    VSGAAAV      R.blastica    LTIQYNRSRQ    AAITKELIEI    ISGAEAL      Bovine    LSLTYNRTRQ    AVITKELIEI    ISGAEAL      PS3    LTLSYNRARQ    AAITGELTEI    VAGAAALQ		296	3 <u>2</u> 2		
Synecnococcus    6301    LTLVYINKARQ    AIAITQUELLEV    VAGALEALNG      E.coli    LQLVYINKARQ    AITQUELTEI    VSGAAAIV      R.blastica    LTIQYNKSRQ    AITKELIEI    ISGAEAL      R.rum    LSLTYNKTRQ    AQITKELIEI    ISGAEAL      Bovine    Bitochondrion    LTLTFNRTRQ    AVITKELIEI    ISGAAAL      PS3    LTLSYNRARQ    AAITQUEITEI    VAGALALL	Spinach chloroplast	LIS IN VINRARQ AKI TGEI LEI	V AIG AINIAIC V		
L  L <td><u>Synechococcus</u> 6301</td> <td></td> <td>V AIG AIEIAIL N G</td> <td></td> <td></td>	<u>Synechococcus</u> 6301		V AIG AIEIAIL N G		
R. rubrum  LSLTYNRTRQ  AQIITKELIEI  ISCALAL    Bovine  mitochondrion  LTLTFNRTRQ  AVITKELIEI  ISGALAL    PS3  LTLSYNRARQ  AAITQEITEI  VAGANALQ	<u>E·COII</u> P. blastica				
Bovine mitochondrion LTLTFNRTRQ AVITKELIEI ISGAAAL PS3 LTLSYNRARQ AAITQEITEI VAGANALQ	R. ruhrum	ISITVNRTRO ANTIKELIEI	I SCAFAL		
PS3 LITLSYNRARQ AAITTQEITEI VAGANALQ	Bovine mitochondrion	ITITENETEG AVITEL			
	PS3	LTLSYNRARQ AAITQEITEI	VAGANALQ		

Fig.2. Alignment of amino acid sequences of  $\gamma$ -subunits from various sources. The sequences of the  $\gamma$ -subunits of *E. coli* [9,10], *R. rubrum* [13], *Rps. blastica* [14], thermophilic bacterium PS3 [15], *Synechococcus* sp. [16], beef heart mitochondria [17], and spinach chloroplasts were aligned to obtain maximal homology. Identical residues are boxed and gaps (--) have been inserted. Amino acid residues of the spinach subunit are numbered from the amino terminus.

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terminal regions of the subunits are highly conserved in the seven species and seem to be essential for assembly and catalysis, consistent with the conclusion drawn from results with E. coli mutants. A nonsense mutant (Gln-269  $\rightarrow$  end) had an F<sub>1</sub> complex without ATPase activity on its membranes, although this complex was unstable after solubilization, whereas another mutant (Gln-261  $\rightarrow$  end) showed essentially no F<sub>1</sub> assembly [12] (E. coli sequence numbered from the second codon [12]). Residues Met-23 and Val-26 of the E. coli  $\gamma$ subunit are conserved in other  $\gamma$ -subunits and this region also contains residues conservatively substituted. Consistent with these findings, a deletion of 7 amino acid residues (Lys-21 to Ala-27) was found to result in loss of  $F_1$  assembly [11]. It will be of interest to replace the conserved residues using site-directed mutagenesis and study the altered function of the mutant subunits.

Consistent with the findings of McCarty and coworkers [23], the chloroplast  $\gamma$ -subunit was shown to have four Cys residues. Cys-89 corresponds to the cysteine residue in peptide S3 [23] that is modified with sulfhydryl reagents such as Nethylmaleimide in the light. The modification resulted in inhibition of ATP synthesis and hydrolysis by  $CF_1$  [24], suggesting that Cys-89 is essential for the CF<sub>1</sub> activity. As shown in fig.2, this Cys residue is conserved in all  $\gamma$ -subunits so far sequenced except that of the thermophilic bacterium PS3 [15], which has an Ala residue in the corresponding position. Four residues around Cys or Ala are conserved in all species, supporting the importance of this region. Cys-322 corresponds to the cysteine residue in peptide S4 [23] and is modified in the dark. Although conserved residues are clustered in the carboxyl-terminal region of the  $\gamma$ -subunits (fig.2), no cysteine was present at the homologous position of the  $\gamma$ -subunits from other sources, suggesting that Cys-322 is not essential for the catalysis. The two Cys residues forming the disulfide bond found in tryptic fragments S1 and S2 [23] correspond to Cys-199 and Cys-205, respectively, of the deduced sequence. The two Cys residues were in the extra-domain (around Ser-193 to Phe-237) which was not found in any other subunits except that of Synechococcus sp. Since reduction of the disulfide bond was associated with activation of ATP hydrolysis and synthesis by  $CF_1$ [7,25], this extra-domain may be essential for the regulation of catalytic activity of  $CF_1$ . In the cyanobacterium, the extra-domain is 9 residues smaller and does not contain the two Cys residues. Further work on the role of the extra-domain in *Synechococcus* sp. appears of interest.

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