Genes Encoding the Beta and Epsilon Subunits of the Proton-Translocating ATPase from *Anabaena* sp. Strain PCC 7120†

STEPHANIE E. CURTIS

Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695-7614

Received 30 June 1986/Accepted 30 September 1986

The genes encoding the β (*atpB*) and ϵ (*atpE*) subunits of the ATPase from the cyanobacterium *Anabaena* sp. strain PCC 7120 were cloned, and their sequences were determined. *atpB* and *atpE* are each single-copy genes in the *Anabaena* genome. The two genes are separated by a 96-base-pair intergenic spacer and transcribed as a single mRNA of 2.3 kilobases that initiates approximately 200 base pairs upstream of the *atpB* coding region. The predicted translation product of *atpB* has 81 and 68% amino acid identity with the corresponding proteins from spinach chloroplasts and *Escherichia coli*, respectively. The *atpE* gene product is less conserved, with 41 and 33% amino acid identity with the corresponding proteins from spinach chloroplasts and *E. coli*, respectively. The organization of the *Anabaena atpB* and *atpE* genes relative to adjacent genes differs from that of both *E. coli* and chloroplasts.

The cyanobacteria are photosynthetic procaryotes capable of an oxygenic photosynthesis virtually identical to that of plant chloroplasts. It has been proposed that the cyanobacteria are related to the evolutionary progenitors of the modern plant chloroplast (15). A proton-translocating ATPase complex of similar structure is found in the chloroplasts and mitochondria of eucaryotic cells, as well as in bacteria (6). Study of the genes encoding the subunits of the ATPase complex thus provides an opportunity to investigate the evolution of an important set of genes across a broad evolutionary scale.

The reversible, proton-translocating ATPase is an enzyme complex that couples ATP synthesis and hydrolysis to proton translocation across a membrane. The complex is composed of two portions, designated F_1 and F_0 , that function in catalysis and proton translocation, respectively. F_1 is an extrinsic membrane protein complex composed of the following five subunits: α , β , γ , δ , and ϵ (6). F_0 is intrinsic to the membrane and is composed of either three or four subunits, depending on the source of the ATPase (2, 6).

The genes encoding the various subunits of the *Escherichia coli* ATPase are tightly linked and form a single transcription unit (6). In contrast, some of the ATPase subunits of chloroplasts and mitochondria are encoded by nuclear genes, while others are encoded by organelle genes. In higher plants, the genes for the α , β , and ϵ subunits of the chloroplast ATPase F_1 (CF_1) are localized on the chloroplast genome, while the δ and γ subunit genes are nuclear (18). The chloroplast CF_1 genes are of higher plants organized at two separate transcription units: the genes encoding the β (*atpB*) and ϵ (*atpE*) subunits are linked and cotranscribed, while the gene encoding the α subunit (*atpA*) maps many kilobase pairs away in a second ATPase gene cluster (18).

In this paper, the structures of the *atpB* and *atpE* genes from the cyanobacterium *Anabaena* sp. strain PCC 7120 are reported. These genes are tightly linked and cotranscribed into a dicistronic mRNA. The β subunit gene is more conserved evolutionarily than is the ϵ subunit gene, and the organization of these two genes relative to adjacent genes differs from that of both *E. coli* and chloroplast genes.

MATERIALS AND METHODS

Isolation of cyanobacterial, phage, and bacterial DNAs. *Anabaena* sp. strain PCC 7120 total cellular DNA was isolated from 15-liter cultures as described previously (3). Lambda bacteriophage and *E. coli* strains were grown, and phage and plasmid DNAs were isolated as described before (20).

Isolation of β and ϵ subunit genes. A recombinant phage library of *EcoRI* fragments of total *Anabaena* DNA cloned in Charon 4A was screened by plaque hybridization as previously described (20) by using a cloned fragment of corn chloroplast DNA. The plasmid pZMB1A (7), kindly provided by A. Gatenby, contains a 4.0-kilobase-pair (kbp) *BamHI* fragment of corn chloroplast DNA that carries the 5' end of the *atpB* gene and the entire gene encoding the large subunit of ribulose biphosphate carboxylase (*rbcL*). pZMB1A DNA was digested with *BamHI*, and the plasmid insert was purified by electroelution from agarose gels. The insert DNA was ^{32}P labeled by nick translation and hybridization with plaque lifts as described previously (20).

Mapping and subcloning of *Anabaena atpB* and *atpE* genes. *Anabaena* DNA inserts were recloned from phage vectors into the plasmid vectors pBR325 and pUC9. Restriction endonuclease digestions, agarose gel electrophoresis, and Southern blot preparations were performed as described previously (20). The *E. coli atpB* gene probe was prepared from the plasmid pRPG55 (8), kindly provided by R. Simoni. The 0.9-kbp insert of pRPG55 was isolated by digestion with *PstI* followed by purification of the insert by electroelution from agarose gels.

Analysis of β and ϵ gene copy number. The number of copies of the *atpB* and *atpE* genes in the *Anabaena* genome was determined by the hybridization of an *atpB-atpE* probe to Southern blots of *Anabaena* cellular DNA digested with *HindIII* and *EcoRI*. A 3.1-kbp *atpB-atpE* probe was generated by the digestion of pAn651 (Fig. 1) with *BamHI* and *PstI*, which cleave outside the insert in the flanking vector DNA. The fragment insert was purified by agarose gel electrophoresis, ^{32}P labeled by nick translation, and hybridized with Southern blots as described previously (20).

DNA sequence determinations. The DNA sequence of the β and ϵ gene regions was determined essentially as described

† Paper no. 10561 of the journal series of the North Carolina Agricultural Research Service.

FIG. 1. Physical map of the region containing the *Anabaena atpB* and *atpE* ATPase genes. The open bars represent the gene coding regions which are separated by 96 bp (Fig. 2). The two genes are transcribed into a dicistronic mRNA (arrow) of 2.3 kb that initiates 200 bp upstream from the *atpB* coding region. A second transcript that maps 65 bp upstream from the *atpB* gene may be derived from the longer transcript or may represent a second transcription start site. A and B indicate the β - and ϵ -specific DNA fragments used in Northern blot hybridizations (see the text).

by Maxam and Gilbert (16). To analyze the sequence of DNA upstream of the *atpB* coding region, the 1.9-kbp *PstI-XbaI* fragment of pAn651 was subcloned into the M13 phage vectors mp18 and mp19. Portions of the 3.1-kbp sequence were determined by the chain termination method (22) with site-directed oligonucleotide primers. The sequences of both strands of the DNA were determined when either method was used. Sequence analyses were performed by using the DNA/Protein Sequence Analysis System computer software (International Biotechnologies, Inc., New Haven, Conn.).

RNA analysis. Total RNA was isolated from *Anabaena* cultures as described previously (25). RNA (30 μ g) was denatured with glyoxal and dimethyl sulfoxide, fractionated on 1% agarose gels, and transferred to nitrocellulose filters as described before (25). Size markers consisting of DNA fragments of known size (1-kb ladder; Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were similarly denatured and fractionated.

atpB and *atpE* probes were prepared from *Anabaena* DNA by the isolation of internal DNA fragments from the plasmid pAn651. An 850-base-pair (bp) *Sau96I-XbaI* fragment from pAn651 was used for the β -specific probe (designated A in Fig. 1), and a 710-bp *DdeI-BamHI* fragment of pAn651 was generated for the ϵ -specific probe (designated B in Fig. 1). All fragments were purified by electroelution from agarose gels, 32 P labeled by nick translation, and hybridized with Northern blots as described previously (25).

Oligonucleotide synthesis. All oligonucleotides were synthesized in an automated DNA synthesizer (model 380A; Applied Biosystems, Inc., Foster City, Calif.). After synthesis, the oligonucleotides were deblocked in concentrated ammonium hydroxide at 60°C for 6 to 12 h and then dried under vacuum. A portion was suspended in 2.5 M ammonium acetate and then precipitated with 3 volumes of cold ethanol at -70°C for 10 min. The precipitate was collected by centrifugation, dried under vacuum, and suspended in water. The solution was desalted by passage through a G-25 Sephadex column. The concentration of the oligonucleotide was calculated from A_{260} measurements.

Primer extension and S1 nuclease assays. Primer extension assays were performed by using an oligonucleotide primer of 18 bases specific to the β gene coding region. Primer (10 ng)

and total *Anabaena* RNA (10 μ g) were combined in 3 μ l of water and boiled for 1 min followed by quick cooling in ice water. Primer extension reactions were carried out in a total volume of 10 μ l containing 100 mM Tris hydrochloride (pH 8.3), 140 mM KCl, 50 mM MgCl₂, 10 mM dithiothreitol, 50 μ M each dGTP, dTTP, and dCTP, 2 μ M [α - 32 P]dATP, and 12 U of reverse transcriptase (Life Sciences, Inc., St. Petersburg, Fla.). These components were added to the boiled nucleic acid mixture and incubated at 37°C. After 30 min, 3 μ l of a chase solution containing 25 mM each dGTP, dCTP, dTTP, and dATP was added, and the mixture was incubated for an additional 30 min at 37°C. The reaction was stopped by the addition of an equal volume of 98% formamide, 0.3% xylene cyanol, and 0.3% bromophenol blue. Samples were boiled for 2 min, and 5- μ l portions were electrophoresed on 5% polyacrylamide gels in the presence of 8 M urea (16). A sequence ladder generated by using the same primer on the appropriate M13 DNA clone was coelectrophoresed and used to determine the position of the transcription start site. S1 nuclease protection assays were performed as described before (25) by using an end-labeled *HinfI* fragment that contains 190 bp upstream of the β initiator codon and ends 620 bp from the initiator codon within the coding region.

RESULTS

Cloning of the *Anabaena atpB* gene. The gene encoding the β subunit of the ATPase was isolated by screening a recombinant lambda library with a corn chloroplast DNA fragment that contains the 5' end of the *atpB* gene. The *Anabaena atpB* gene was localized to an *EcoRI* fragment of 5.6 kbp.

The 5.6-kbp *EcoRI atpB* gene fragment was subcloned into plasmid vector pBR325 to generate the plasmid pAn650 (Fig. 1). By restriction endonuclease mapping and Southern blot analysis with an *atpB* probe from *E. coli*, the *atpB* gene was further localized to a 3.1-kbp *HpaI* fragment (Fig. 1). This fragment was blunt-end ligated into a *HincII* digest of the plasmid pUC9 to generate the plasmid pAn651. Hybridization of the 3.1-kbp *HpaI* fragment containing *atpB* and *atpE* to *Anabaena* DNA cleaved with enzymes which cut outside the *HpaI* fragment gave a single band of predicted size, indicating that these genes are single copy (data not shown).

DNA sequence analysis of the *atpB* gene and flanking regions. Determination of the DNA sequence of the entire 3.1-kbp *HpaI* fragment demonstrated that the fragment contained the complete *atpB* coding region (Fig. 2). Analysis of the region flanking the 3' end of the *atpB* gene revealed an open reading frame that commences 96 bp downstream from the *atpB* coding region (Fig. 2). This open reading frame shares homology with the *atpE* genes of *E. coli* and spinach chloroplasts (see below). Based on this homology and position downstream from the *atpB* gene, the second smaller open reading frame is identified as the *Anabaena atpE* gene. Determination of the DNA sequence 1.0 kbp upstream from the *atpB* gene indicated that this region contains an open reading frame of greater than 600 bp with no detectable homology to other ATPase gene products.

Analysis of *atpB* and *atpE* transcripts. DNA fragments specific to the *atpB* and *atpE* genes hybridized with a single mRNA species of 2.3 kilobases (kb) (Fig. 3). To define the transcription unit more precisely, the 5' end of *atpB* transcript was mapped. An 18-base synthetic oligonucleotide specific to the beta coding region was annealed with total RNA and extended in a primer extension assay (Fig. 4). Two

FIG. 2. Nucleotide sequence of the noncoding strand of the *atpB* and *atpE* genes and flanking regions. The β and ϵ coding regions are boxed. The two arrowheads indicate the 5' ends of mRNA transcripts. Potential ribosome binding site sequences complementary to the 3' end of the 16S rRNA of *Anacystis nidulans* are underlined. Arrows represent two clusters of different tandem direct repeats.

extended products that map to 65 and 200 bp upstream from the *atpB* coding region were observed (Fig. 2 and 4). This result was confirmed in an S1 nuclease protection assay (data not shown). Since the β transcript initiated close to the *atpB* coding region and was 2.3 kb in length, the transcript must also contain *atpE* gene sequences 3' to the *atpB* gene.

Interspecies comparisons of the *atpB* and *atpE* genes. The *atpB* and *atpE* genes encode proteins with an M_r of 50,797 and 14,670 (Fig. 5), respectively, in good agreement with the molecular weights determined by denaturing sodium dodecyl sulfate gel electrophoresis for the β and ϵ subunits of the ATPase from the cyanobacterium *Spirulina platensis* (9). The amino acid sequences of the corresponding proteins from *E. coli* (13, 23) and spinach chloroplasts (30) are shown in comparison in Fig. 5, aligned to display maximum homology. The β protein comparison demonstrates a high degree of interspecies conservation for this subunit of the ATPase.

The *Anabaena* beta protein has 68 and 81% amino acid identity with the corresponding proteins from *E. coli* and spinach chloroplasts, respectively. Particularly striking in the comparisons are two stretches (residues 284 to 303 and 429 to 450), in which the amino acid sequences of the three species are identical. Although the overall conservation is high, there is heterogeneity in the length of the protein due to variation at internal positions and at both the carboxyl and amino termini. In two internal regions, the derived spinach chloroplast β protein sequence has extra amino acid residues relative to the *E. coli* protein. In one of these regions, residues 54 to 58, the *Anabaena* protein has the same number of amino acids as the chloroplast protein. In the second region, residues 223 to 230, the *Anabaena* protein has 4 fewer amino acids than the *E. coli* protein, and 11 fewer than the chloroplast protein.

The ϵ subunit of the ATPase displays greater divergence

FIG. 3. Identification of the *atpB* and *atpE* mRNA species. Total *Anabaena* RNA (20 μ g) was denatured, electrophoresed, transferred to nitrocellulose paper, and hybridized with *atpB*- or *atpE*-specific probes (see the text). Numbers at left indicate the sizes (kb) of DNA fragments used as molecular weight standards.

than does the β protein relative to the spinach and *E. coli* proteins. The *Anabaena* protein has 31 and 44% amino acid identity with the proteins from *E. coli* (13) and spinach chloroplasts (30), respectively (Fig. 5B). As with the β protein, there is length variation in both internal and terminal regions.

DISCUSSION

Organization and expression of *atpB* and *atpE*. The genes encoding the β and ϵ subunits of the proton-translocating ATPase from *Anabaena* sp. are linked and cotranscribed, analogous to the arrangement in the chloroplasts of higher plants (18) and *E. coli* (6). In several chloroplast genomes, the two genes overlap by a few nucleotides (18). The *Anabaena atpB* and *atpE* gene spacing is more similar to that of *E. coli*, in which a spacer region separates the two genes. The *Anabaena* spacer is four times as long as that of *E. coli* (96 versus 23 bp) and contains numerous short direct repeats (Fig. 2) that are presumably the result of duplication events. In contrast to the overall organization of ATPase genes in *E. coli*, the *Anabaena atpE* and *atpB* genes are not closely linked to other ATPase genes. The region upstream from the *atpB* gene contains an open reading frame whose identity is unknown. This region does not share homology with other ATPase genes. The genes for the remaining *Anabaena* ATPase subunits map in a gene cluster that is at least 2 kb from the *atpB* and *atpE* genes (D. F. McCarn and S. E. Curtis, unpublished data). This arrangement of ATPase genes, also seen in the cyanobacterium *Synechococcus* sp. strain PCC 6301 (2), is reminiscent of plant chloroplast genomes in which the linked *atpB* and *atpE* genes map many kilobase pairs away from the other ATPase subunit genes.

However, unlike the organization of genes in many chloroplast genomes (18), the *Anabaena rbcL* gene does not map in the region just upstream of the *atpB* gene (3). The arrangement of ATPase subunit genes of *Anabaena* sp. is thus intermediate between the arrangement observed in *E. coli* and chloroplasts.

The *atpB* and *atpE* genes are expressed on an mRNA of approximately 2.3 kb. The endpoints of two RNA transcripts that differ by 135 bp have been mapped upstream of the *atpB* gene. It is not known whether the shorter transcript is derived from the longer by processing or degradation, or whether the transcripts represent distinct transcription initiation sites. The position of the longer transcript endpoint is preceded by sequences that resemble an *E. coli* promoter sequence (19). Thus far, a consensus sequence for *Anabaena* promoters utilized during growth on complete medium has not been identified. The transcription initiation site identified for one other *Anabaena* gene is preceded by an *E. coli*-like promoter (25), while the transcription initiation sites for other genes are not preceded by such sequences (3, 17).

The sequence of the 3' end of the *Anabaena* 16S rRNA has not been determined but is expected to be similar to that of *Anacystis nidulans*. The 3' end of the *Anacystis* 16S rRNA has the same sequence as the mRNA binding region of *E. coli* 16S rRNA (1), and thus cyanobacterial ribosome binding sequences are expected to be similar to the *E. coli* Shine-Dalgarno sequence (24). The *atpB* and *atpE* genes do not have typical ribosome binding sequences (24) upstream from the coding regions. However, in each case there is a triplet that is complementary to the 3' end of the 16S rRNA from

FIG. 4. Identification of start sites for *atpB* transcription. An 18-nucleotide primer specific to the 5' end of the beta coding region was used in a primer extension assay with total *Anabaena* RNA. The extended products (P) were electrophoresed in parallel with a sequence ladder (G, A, C, T) generated by using the same primer on the noncoding strand of the DNA.

The proteins from *Anabaena* sp. and spinach chloroplasts are about equally similar to the *E. coli* β protein, with 68 and

Photoaffinity labeling of the bovine mitochondrial F_1 with

8-azido-ATP (11) results in the predominant labeling of three residues of the β subunit (corresponding to the spinach residues 317, 321, and 328) (Fig. 5) which may be in the vicinity of the ATP binding site. These residues are conserved in *E. coli*, *Anabaena* sp., and spinach chloroplasts, with the exception of position 317 in *Anabaena* which is a threonine residue rather than a lysine residue.

In contrast to the β subunit, the ϵ subunit primary sequence is not so highly conserved. The *Anabaena* ϵ protein is intermediate in sequence homology between the epsilon subunits from bacteria and chloroplasts, sharing 41 and 33% amino acid identities with the proteins from spinach chloroplasts and *E. coli*, respectively. The spinach chloroplast and *E. coli* proteins share 26% amino acid identities (30). Consistent with the relatively low degree of homology between the chloroplast and *Anabaena* sequences is the lack of interaction between antibodies to spinach ϵ and the ϵ subunit from the cyanobacterium *Spirulina platensis* (10a). The ϵ subunits from chloroplasts (21) and *E. coli* (14) have both been shown to be inhibitors of ATPase activity. If this inhibitor function is conserved among ϵ subunits, it is not possible to correlate functionally important regions with sequences conserved among chloroplasts, *E. coli*, and cyanobacteria.

ACKNOWLEDGMENTS

Loucinda Hoy provided excellent technical assistance during the course of this work. I thank A. Gatenby and R. Simoni for ATPase DNA probes, J. Alam for the synthesis of oligonucleotide primers, and R. Swanson for helpful suggestions on both experimental design and manuscript preparation.

This work was supported by Public Health Service grant GM2766 from the National Institutes of Health and North Carolina Biotechnology Center grant 84-3352.

LITERATURE CITED

- Borbely, G., and A. Simoncsits. 1981. 3'-Terminal conserved loops of 16S rRNAs from the cyanobacterium *Synechococcus* PCC 6301 and maize chloroplast differ only in two bases. *Biochem. Biophys. Res. Commun.* **101**:846-852.
- Cozens, A. L., J. E. Walker, A. L. Phillips, A. K. Huttly, and J. C. Gray. 1986. A sixth subunit of ATP synthase, an F_0 component, is encoded in the pea chloroplast genome. *EMBO J.* **5**:217-222.
- Curtis, S. E., and R. Haselkorn. 1983. Isolation and sequence of the gene for the large subunit of ribulose-1,5-bisphosphate carboxylase from the cyanobacterium *Anabaena* 7120. *Proc. Natl. Acad. Sci. USA* **80**:1835-1839.
- Curtis, S. E., and R. Haselkorn. 1984. Isolation, sequence and expression of two members of the 32kd thylakoid membrane protein gene family from the cyanobacterium *Anabaena* 7120. *Plant Mol. Biol.* **3**:249-258.
- Esch, F. S., P. Bohlen, A. S. Otsuka, M. Yoshida, and W. S. Allison. 1981. Inactivation of the bovine mitochondrial F_1 -ATPase with dicyclohexyl [14 C]carbodiimide leads to the modification of a specific glutamic acid residue in the β subunit. *J. Biol. Chem.* **256**:9084-9089.
- Futai, M., and H. Kanazawa. 1983. Structure and function of proton-translocating adenosine triphosphatase (F_0F_1): biochemical and molecular biological approaches. *Microbiol. Rev.* **47**:285-312.
- Gatenby, A. A., J. A. Castleton, and M. W. Saul. 1981. Expression in *E. coli* of maize and wheat chloroplast genes for large subunit of ribulose bisphosphate carboxylase. *Nature (London)* **291**:117-121.
- Gunsalus, R. P., W. S. A. Brusilow, and R. D. Simoni. 1982. Gene order and gene-polypeptide relationships of the proton-translocating ATPase operon (*unc*) of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **79**:320-324.
- Hicks, D. B., and C. F. Yocum. 1986. Properties of the cyanobacterial coupling factor ATPase from *Spirulina platensis*. I. Electrophoretic characterization and reconstitution of photophosphorylation. *Arch. Biochem. Biophys.* **245**:220-229.
- Hicks, D. B., and C. F. Yocum. 1986. Properties of the cyanobacterial coupling factor ATPase from *Spirulina platensis*. II. Activity of the purified and membrane-bound enzymes. *Arch. Biochem. Biophys.* **245**:230-237.
- Hicks, D. B., and C. F. Yocum. 1986. Cyanobacterial and chloroplast F_1 -ATPases: cross-reconstitution of photophosphorylation and subunit immunological relationships. *Biochim. Biophys. Acta* **851**:217-222.
- Hollemans, M., M. J. Runswick, I. M. Fearnley, and J. E. Walker. 1983. The sites of labeling of the β -subunit of bovine mitochondrial F_1 -ATPase with 8-azido-ATP. *J. Biol. Chem.* **258**:9307-9313.
- Howe, C. J., I. M. Fearnley, J. E. Walker, T. A. Dyer, and J. C. Gray. 1985. Nucleotide sequences of the genes for the alpha, beta and epsilon subunits of wheat chloroplast ATP synthase. *Plant Mol. Biol.* **4**:333-345.
- Kanazawa, H., T. Kayano, T. Kiyasu, and M. Futai. 1982. Nucleotide sequence of the genes for β and ϵ subunits of proton-translocating ATPase from *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **105**:1257-1264.
- Klionsky, D. J., W. S. A. Brusilow, and R. D. Simoni. 1984. In vivo evidence for the role of the ϵ subunit as an inhibitor of the proton-translocating ATPase of *Escherichia coli*. *J. Bacteriol.* **160**:1055-1060.
- Margulis, L. 1981. Symbiosis in cell evolution, p. 37-62. W. H. Freeman & Co., San Francisco.
- Maxam, A. M., and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65**:499-559.
- Nierzwicki-Bauer, S. A., S. E. Curtis, and R. Haselkorn. 1984. Cotranscription of gene encoding the small and large subunits of ribulose-1,5-bisphosphate carboxylase in the cyanobacterium *Anabaena* 7120. *Proc. Natl. Acad. Sci. USA* **81**:5961-5965.
- Palmer, J. D. 1983. Comparative organization of chloroplast genomes. *Annu. Rev. Genet.* **19**:325-354.
- Reznikoff, W. S., D. A. Siegle, D. W. Cowing, and C. A. Gross. 1985. The regulation of transcription initiation in bacteria. *Annu. Rev. Genet.* **9**:355-388.
- Rice, D., B. J. Mazur, and R. Haselkorn. 1982. Isolation and physical mapping of nitrogen fixation genes from the cyanobacterium *Anabaena* 7120. *J. Biol. Chem.* **257**:13157-13163.
- Richter, M. L., M. L. Patrie, and R. E. McCarty. 1984. Preparation of the epsilon subunit-deficient chloroplast coupling factor 1 in reconstitutionally active forms. *J. Biol. Chem.* **259**:7371-7373.
- Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-termination inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463-5467.
- Saraste, M., N. J. Gay, A. Eberle, M. J. Runswick, and J. E. Walker. 1981. The *atp* operon: nucleotide sequence of the genes for the γ , β , and ϵ subunits of *Escherichia coli* ATP synthase. *Nucleic Acids Res.* **9**:5286-5296.
- Shine, J., and L. Dalgarno. 1974. The 3'-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to non-sense triplets and ribosome binding sites. *Proc. Natl. Acad. Sci. USA* **71**:1342-1346.
- Tumer, N. E., S. J. Robinson, and R. Haselkorn. 1983. Different promoters for the *Anabaena* glutamine synthetase gene during growth using molecular or fixed nitrogen. *Nature (London)* **306**:337-341.
- Walker, J. E., A. Eberle, N. J. Gay, M. J. Runswick, and M. Saraste. 1982. Conservation of structure in proton-translocating ATPases of *Escherichia coli* and mitochondria. *Biochem. Soc. Trans.* **10**:203-206.
- Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay. 1982. Distantly related sequences in the α - and β -subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes

- and a common nucleotide binding fold. EMBO J. 1:945-951.
28. Yoshida, M., W. S. Allison, F. S. Esch, and M. Futai. 1982. The specificity of carboxyl group modification during the inactivation of the *Escherichia coli* F₁-ATPase with dicyclohexyl ¹⁴C-carbodiimide. J. Biol. Chem. 257:10033-10037.
29. Yoshida, M., J. W. Poser, W. S. Allison, and F. S. Esch. 1981. Identification of an essential glutamic acid residue in the β subunit of the adenosine triphosphatase from the thermophilic bacterium PS3. J. Biol. Chem. 256:148-153.
30. Zurawski, G., W. Bottomley, and P. R. Whittfeld. 1982. Structures of the genes for the β and ϵ subunits of spinach chloroplast ATPase indicate a dicistronic mRNA and an overlapping translation stop/start signal. Proc. Natl. Acad. Sci. USA 79: 6260-6264.