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Short Communication

Ribosomal Proteins in the Cyanobacterium *Anabaena variabilis* Strain M3: Presence of L25 Protein

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Ribosomal proteins from a cyanobacterium *Anabaena variabilis* were analyzed by two-dimensional gel electrophoresis. We detected 21 protein spots of the small subunit and 29 protein spots of the large subunit. One of the spots was identified as L25 protein, which suggests that the reading frame sll1824 of *Synechocystis* is the L25 protein.

Key words: Cyanobacterium — 50S ribosomal protein L25 — Ribosome.

The ribosome is the central machinery of protein synthesis. The complete amino acid sequences of the ribosomal proteins (35 proteins of the large subunit and 21 proteins of the small subunit of *E. coli* were established in the early 1980s (Wittmann 1982, Wada and Sako 1987), but the molecular details of the ribosomal proteins of other prokaryotes remained unclear until the recent determination of genomic sequences of various prokaryotes. Now the DNA sequence information of ribosomal protein genes is rapidly accumulating. In spite of this progress, little is known about the actual composition and structure of prokaryotic ribosomes at the protein level. In *Synechocystis* PCC 6803, all of the 21 genes for the proteins of the small subunit have been identified, while only 31 genes for the proteins of the large subunit have been reported (Kaneko et al. 1996). Furthermore, comparison of the genome sequences of various prokaryotes that were recently sequenced reveals a small variation of the protein composition of the two subunits. In *Bacillus subtilis* (Kunst et al. 1997) and *Mycoplasma genitalium* (Fraser et al. 1995), *rpsI* is missing; in *B. subtilis, Synechocystis* sp. PCC6803, and *M. genitalium, rpl25* is reported to be missing, while *rpl30* is lacking in *Helicobacter pylori* (Tomb et al. 1997), *Synechocystis* sp. PCC6803 and *M. genitalium. Rps21* is also absent in *M. genitalium*.

During the course of a study on the temperature-dependent regulation of the *rpsU* (*rps21*) gene in *Anabaena variabilis* (Sato et al. 1997), we analyzed the ribosomal proteins of this cyanobacterium. The aim of the present study is to obtain a general view of the ribosomal proteins of *Anabaena* by sequence analysis of the separated proteins, with special attention to the presumptive missing components as mentioned above.

Anabaena variabilis strain M3 (identical to the strain PCC7118 in the origin) was obtained from the culture collection of the University of Tokyo, and was grown at 22°C as described (Kratz and Myers 1955, Sato 1994, 1995, Sato

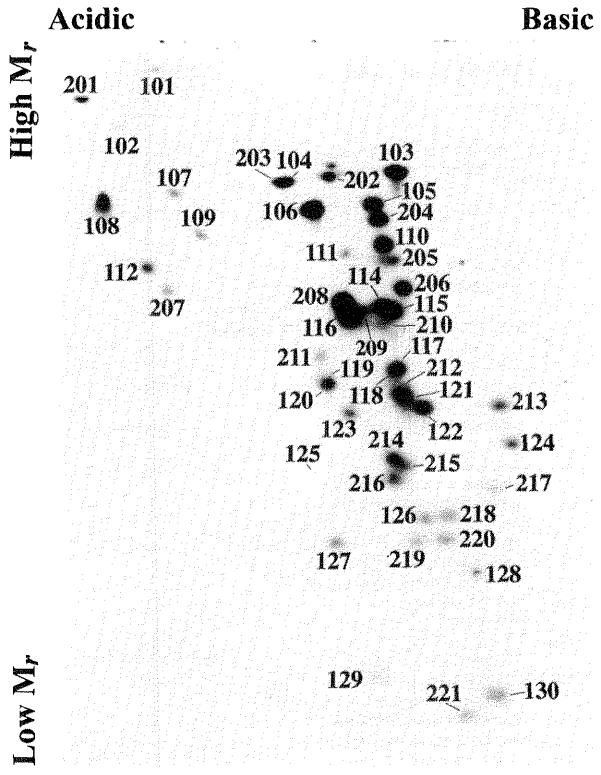

Fig. 1 Two-dimensional electrophoretic analysis of the ribosomal proteins of *A. variabilis*. The spots of the large subunit are numbered from 101 to 130 (number 113 is missing), whereas the spots of the small subunits are numbered from 201 to 221. The spot 123 was found to correspond to L25, while the spot 205 corresponds to S5.

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et al. 1997). Two-dimensional analysis of ribosomal proteins was performed essentially as described previously (Sato et al. 1997). The 70 S ribosomes were prepared from the cells by differential centrifugation. Ribosomal proteins were extracted by the acetic acid method (Hardy et al. 1969) and then analyzed by the radical-free and highly reducing (RFHR) method of two-dimensional gel electrophoresis (Wada 1986). The gel was stained with Amido Black 10B and then subjected to densitometry (Wada et al. 1993). For the analysis of N-terminal sequences, the separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane, and each protein spot was sequenced on Applied Biosystems 477A sequencer. Sequences of ribosomal proteins of various prokaryotes were retrieved from GenBank and SWISS-PROT detabases mirrored at Genome Net in Japan. Multiple alignments of sequences of individual ribosomal proteins were constructed by the CLUSTAL W program (Thompson et al. 1994) for UNIX on a Silicon Graphics workstation model O2 (180 MHz) or a Linux workstation with a Pentium processor (120 MHz). The N-terminal sequences of ribosomal proteins of *A. variabilis* were compared with these multiple alignments by CLUSTAL W program in the profile alignment mode. To complement this analysis, a BLAST search of the N-terminal sequences with the protein database of *Synechocystis* sp. PCC6803 was performed. For this purpose, either the BLAST server at Cyanobase (http://www.kazusa.or.jp) or a BLAST2 program (Altschul et al. 1997) running locally on a Linux workstation were used.

Figure 1 shows a typical result of two-dimensional

Table 1 Assignment of ribosomal proteins

Spot	Sequence	Assignment
	-- Large subunit --	
101	(ND)	
102	(ND)	
103	xTxxxYxxYTPSTRXVXI	L2
	MGIRNYRPMTPGTRQASV	
104	TKRVKRRLRELQAKVEDR-EY	L1
	MTKKLSKRMQAAIAKVDDSKLY	
105	SVGILGTKLGMTEIFD-EAGV	L3
	MSIGILGTKLGMTQIFDQESGI	
106	WxTxLKTLYQETIVPKLF	L5
	MTQRLKTLYQETILPKLQ	
107	(ND)	
108	(ND)	
109	(ND)	
110	MRIxKRPITVPAKVQxQIDD	L6
	MSRIGKRPIPLPAKVSVDIQG	
111	(ND)	
112	DLIYILTGGKQVxxxPKxFY	L21
	MSYAIIEIGGTQIRVEPGRFY	
114	(ND)	
115	MRLNNVKPQKNxxKRxxRVG	L15
	MNLSELSPKDGAKKRRRVG	
116	AIxPLKYxRIARNxNMIP	L22(?)
	MTKLDTTAEVKAIARYVRMSPLKVRRVLDQIRGRS	
117	SAQNIIRKIxAxQxKSNxPL	L19
	MIMNAQAIINSIEAEFLKEDLPT	
118	AKFYPxYLRRLHVRRIxxNG	L18
	MKSTRKSATQRRHRRLRRHL	
119	RxLTNGERxEERxRGxxxGS	unknown
120	(ND)	
121	PSxAVQQG	unknown
122	MRHRNRVKKLGKPADQRRAL	L17
	MRHRCRVPQLGKPADQRKAL	
123	ALTVETKKRPIGSKPxALRRVGFILNxAPY	L25
	MALSIECQQRPEKVNPRALRREGLIPATLYG	(sll1824)
124	TxVKRxNVARKDRNKILKLA	L20
	MTRVKRGNVARKRRKKILKLA	
125	MKSxIxPKxY	L31
	MPKADIHPTWY	
126	AxxKGTGSTI	L27
	MAHKKGTGSTI	
127	QLTPIIxAxLLSAERLVExI	L29
	MALPNIADARKLGDEELATEI	
128	MxNx-DLTGNQANNAFAVS	L28
	MARRCQLTGKKANNGFAVS	
129	AVPKKKTSKxKxDKxNATT	L32
	MAVPKKKTSKAKRDQRRAHW	
130	AKxPTAKAIVTLFxATGWGK	L35
	MPKLKTRKAAAKRFRPTGSGK	

Spot	Sequence	Assignment
	-- Small subunit --	
201	(ND)	
202	GTxIxPV	S3
	MGQKIHPV	
203	(ND)	
204	SRYRGPRLPIVxRLGDxP	S4
	MSRYRGPRLRIVRRLGELP	
205	MGxRRKANRTKxRETNTT	S5
	MAKRRKTSREKKEDTNWQ	
206	SxRGVIQRRPVPPPDSVYN	S7
	MSRRGNVKKRPVPPDPVYN	
207	(ND)	
208	xATYTIAKMLAxIRNAVMA	S8
	MASTDTISDMLTRIRNACAV	
209	AxAPANSxRAVYxGR	S9
	MQANDSSNKVVYWGT	
210	ARQPRKKxSRKKxKRNVPxG	S11
	MARPTRKTGPKKA-KKNIPVG	
211	(ND)	
212	AxIAGVDLPPPKDRVRIxLY	S13
	MARIAGVDLPRDKRVEIALTY	
213	PTIQQLIRTxREKARQKTKS	S12
	MPTIQQLIRSERSKVQKKTKS	
214	AKKSMIEREKTRAYLVYKYS	S14
	MAKKSMIERDKRRSRLVAKYA	
215	MIKLRLKRFIKKREA	S16
	MIKLRLKRFGKKREV	
216	ALTQQRKQxIINNYQVHGT	S15
	MSLTQIRKQELMTEYQAHET	
217	ANNKSALKxAQIAxRxRV	S20
	MANIKSALKRIEIAERNRL	
218	GCSLKKGPFIADHLLSKIEK	S19
	MGRSLKKGPFVAASLLRKIDK	
219	SYYRRRLSPIK	S18
	MNYYRKRLSPLP	
220	AIxERVGLVVSDKMQKN	S17
	MAIKERVGIVVSNKMDKT	
221	TQKVVGENEHIESALRRF	S21
	MTQIVVGENEHIESALRRF (AVA)	
	MTQVVVGQNEPIESALRRF (SYN)	

Unidentified proteins: L4, L9, L10, L11, L7=L12, L13, L14, L16, L23, L24, L33, L34, L36, S1, S2, S6 and S10.
ND, not determined (blocked). In each alignment, the upper sequence is N-terminal sequence of *Anabaena* ribosomal protein, whereas the lower one is *Synechocystis* sequence. In the case of 221, *Anabaena* sequence deduced from gene sequence is also presented (AVA). Bold letters indicate residues conserved between *A. variabilis* and *Synechocystis* sp. PCC6803.

analysis of the ribosomal proteins of purified 70S ribosomes of *A. variabilis*. Because the proteins were stained with Amido Black 10B, proteins with a higher molecular mass and higher isoelectric point were stained more intensely. The distribution of the protein spots in the large and the small subunits was estimated after dissociation of the two subunits according to the standard procedure. In this figure, the protein spots of the large subunit are tentatively numbered from 101 to 130 (number 113 is missing in the last version of result), while the spots of the small subunit are tentatively numbered from 201 to 221. To avoid possible confusion, we should emphasize that these numbers have nothing to do with the finally assigned ribosomal protein name.

The N-terminal sequence of each spot was analyzed and summarized in Table 1. Among the 29 proteins of the large subunit, the amino terminal sequences of eight proteins were not determined because they were blocked. Four of the 21 proteins of the small subunit were likewise found to be blocked. The determined sequences were compared with known ribosomal sequences. For this purpose, all of the known sequences of prokaryotic and plastid ribosomal proteins were retrieved from the GenBank and SWISS-PROT databases. Then, alignments of individual proteins were constructed by the CLUSTAL W program. During this step, some of the proteins, such as L23, L24, and S5, were difficult to align, due to variation of the N- and C-terminal sequences. In these cases, we made alignments of sequences of only the *Synechocystis* protein and some plastid-encoded algal proteins. Finally, these alignments were compared with each of the N-terminal sequences of the *Anabaena* ribosomal proteins. In many cases, a simple BLAST search (Altshul et al. 1997) of the N-terminal sequence with the amino acid database of *Synechocystis* was successful, but about 10 proteins were identified by manual comparison with the alignments of known sequences.

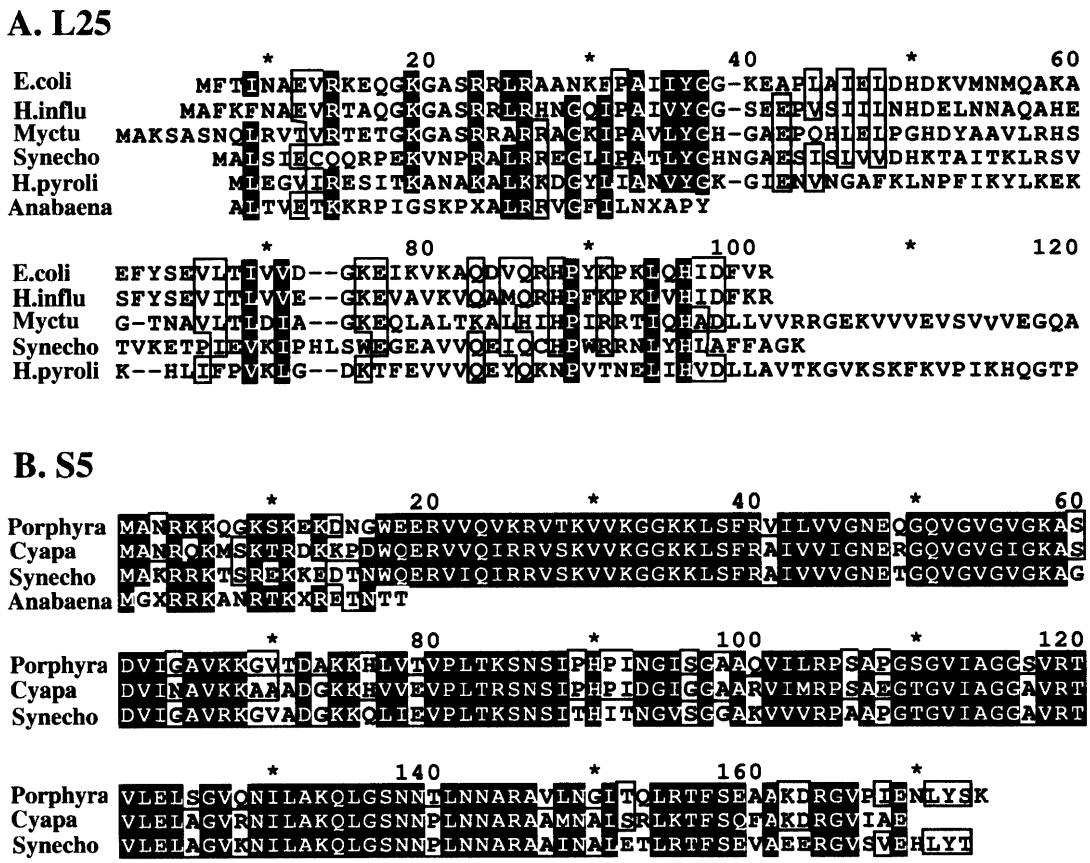

Fig. 2 Alignment of the amino acid sequences. A. L25 proteins. Sources: *Escherichia coli* (E. coli), P02426; *Haemophilus influenzae* (H. influ), P45281; *Mycobacterium tuberculosis* (Myctu), P96385; *Synechocystis* sp. PCC6803 (Synecho), sll1824; *Helicobacter pyroli* (H. pyroli), P56078. The residues that are conserved in 5 sequences are highlighted by the dark background. The residues that are conserved in 4 sequences are boxed. In all cases, conservative substitutions such as V, I and L were allowed. The sequences of *M. tuberculosis* and *H. pyroli* are truncated arbitrarily at residue 120 for simplicity. B. S5 proteins. Sources: *Porphyra purpurea* plastid genome (Porphyra), P51298; *Cyanophora paradoxa* (Cyapa) plastid genome, P23402; *Synechocystis* sp. PCC6803 (Synecho), P73304 or sll1812. The residues that are conserved in 3 and 2 sequences are highlighted by dark background and boxes, respectively.

Table 1 shows the assignment of the ribosomal proteins as well as the alignment of the N-terminal sequence with the *Synechocystis* sequence. Because, for most proteins, the *Synechocystis* sequence was the most similar to the *Anabaena* sequence, only the *Synechocystis* sequence is presented in this table for comparison. We were not able to identify the spots 119 and 121. The spot 116 was tentatively identified as L22, but the N-terminal sequence determined for *A. variabilis* corresponded to the sequence beginning at the 18-th residue of *Synechocystis* L22. This might be explained by the processing of the N-terminus, or use of the second methionine for translational initiation in the *Synechocystis rpl22* gene. The spot 123 was similar to the internal sequence of an unidentified reading frame sll1824 of *Synechocystis*, as well as to various L25 proteins. A close examination of the sll1824 sequence (in D90905) indicated that the first half of this reading frame overlapped with an upstream reading frame, and that the methionine 88 seemed to be the correct initiation codon. The alignment of L25 proteins (Fig. 2A) indicates that the N-terminal sequence of spot 123 of *A. variabilis* matches the N-terminal sequence of this new reading frame of *Synechocystis*. Therefore, L25 is present in both *Synechocystis* and *Anabaena*. Curiously, *rpl25* has not been found in either the genome of *B. subtilis* or that of *M. genitalium*. If L25 were erroneously believed to be absent in *Synechocystis* according to the Cyanobase, a serious misunderstanding about a similarity of the *Synechocystis* ribosome with the ribosomes of *B. subtilis* and *M. genitalium* might have been made. Therefore, it is important to point out that L25 is present in cyanobacteria.

The S5 proteins of various organisms are highly divergent in their N- and C-terminal sequences. For example, the S5 protein of *M. genitalium* contained a long N-terminal extra sequence. We, therefore, made an alignment of the sequence of *Synechocystis* sp. PCC6803 with the plastid sequences. The N-terminal sequence of *A. variabilis* S5 protein matched exactly the N-termini of these homologs (Fig. 2B).

The *rpl30* gene is not present in *B. subtilis, Synechocystis* sp. PCC6803, and *M. genitalium*. The N-terminal analysis of the ribosomal proteins of *A. variabilis* supports the lack of L30 in cyanobacteria.

We finally identified 19 proteins of the large subunit and 17 proteins of the small subunit. Unidentified proteins include: L4, L9, L10, L11, L7=L12, L13, L14, L16, L23, L24, L33, L34, L36, S1, S2, S6 and S10. Although no sequence data is available, the dense spot that we numbered 108 is likely to be L7=L12, since four copies of this protein exist per ribosome in *E. coli*. Several proteins might be either blocked at their N-terminus or divergent in the N-terminal sequence, and escaped identification in the present study. In summary, we emphasize the importance of confirmation of the results of genomic sequencing by protein sequencing.

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