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Effect of temperature on natural mRNA-directed protein synthesis in psychrophilic and mesophilic bacteria

Akinobu OSHIMA*, Noriyuki FUKUNAGA and Shoji SASAKI**

Temperature-dependency of elongation and initiation steps during translation was compared between *Pseudomonas aeruginosa*, a mesophile, and *Vibrio* sp. strain ABE-1, a psychrophile, in endogenous mRNA system and purified polysome system. In the endogenous mRNA system, the relative rate of protein synthesis of *Vibrio* ABE-1 was much higher than that of *P. aeruginosa* below 25° C. This is consistent with the previous results obtained with the poly(U)-directed polyphenylalanine-synthesizing systems. In purified polysome system, amino acid incorporation into proteins and composition of ribosomal fraction in the presence or absence of aurintricarboxylic acid (ATA) suggested that the initiation of translation was blocked in *P. aeruginosa* at low temperature, but not in *Vibrio* ABE-1. This *in vitro* block of the initiation was not relieved by addition of crude initiation factors or ribosomes of *Vibrio* ABE-1.

We presume that the protein-synthesizing systems of psychrophilic and psychrotrophic bacteria ought to function sufficiently as to support their growth even at low temperature. We found that the protein-synthesizing activity in intact cells of a psychrophilic bacterium, *Vibrio* sp. strain ABE-1, was increased about 4-fold after chilling at 0° C for 12 h (SARUYAMA *et al.*, 1980), and that poly(U)-directed polyphenylalanine synthesis in a cell-free system of *Vibrio* ABE-1 was higher than that in *Pseudomonas aeruginosa* system at lower temperatures (OSHIMA *et al.*, 1980). However, the mechanisms by which proteins are actively synthesized in psychrophiles at low temperature have scarcely been elucidated. In this respect, SZER reported the existence of an activation factor, P-factor, in a psychrophilic bacterium, *Pseudomonas* 412 (SZER, 1970), and we reported that the ribosomal 50S subunits of *Vibrio* ABE-1 contribute to the high poly(U)-directed polyphenylalanine synthesis (OSHIMA *et al.*, 1980).

On the other hand, the reason that the protein synthesis in mesophilic

* Present address: Department of Biology, Faculty of Science, Shimane University, Matsue 690, Japan

** To whom correspondence should be addressed.

bacteria is repressed by low temperature has been regarded as the results of the disturbance of the elongation-termination or initiation step of translation (SARUYAMA *et al.*, 1980; BROEZE *et al.*, 1978). Therefore, an attempt was made to examine the effects of temperature on such a step of translation in *Vibrio* ABE-1 and *P. aeruginosa* with natural mRNA-directed protein-synthesizing systems.

In this paper, we report the difference between the psychrophile and mesophile in the initiation step of the translation at low temperature.

Materials and Methods

Cultivation of organisms

Escherichia coli Q13, a laboratory strain of *Pseudomonas aeruginosa*, and *Vibrio* ABE-1 (TAKADA *et al.*, 1979) were cultivated at 37°, 20° and 10° C, respectively, by vigorous shaking in a medium consisting of 1 % each of peptone and meat extract (*E. coli* Q13 and *P. aeruginosa*) and in the same medium containing 3 % NaCl (*Vibrio* ABE-1).

Preparation of components for protein synthesis

Cells in the early exponential phase were chilled on crushed ice and washed three times with Buffer I consisting of 20 mM Tris-HCl, pH 7.8, 60 mM NH_4Cl, 10 mM magnesium acetate, and 6 mM 2-mercaptoethanol. In the case of *Vibrio* ABE-1, the same buffer containing 3 % NaCl was used. Washed cells were ruptured by the method of RON *et al.* (1966) with a minor modification. Crude lysate was centrifuged at $30,000 \times g$ for 30 min and the resultant supernatant was used as crude S-30 fraction. The crude S-30 fraction was layered on the sucrose step gradient consisting of 4 ml each of 0.4 and 1.2 M sucrose in a Beckman # 65 rotar and centrifuged at $150,000 \times g$ for 80 min at 1° C. The pellets were resuspended in Buffer I and the suspension was used as purified polysome fraction. Ribosomes and supernatant fraction were prepared from *E. coli* Q13, *P. aeruginosa* and *Vibrio* ABE-1 as described previously (OSHIMA *et al.*, 1980). Crude initiation factors were prepared from the high-salt ribosomal wash by the method of OHTA *et al.* (1967).

Preparation of R17 RNA

RNA-phage R17 was grown and purified (GESTELAND and BOEDTKER, 1964), then R17 RNA was prepared from phage by phenol extraction followed by alcohol precipitation according to the method described by WEBSTER *et al.* (1967). The precipitate was lyophilized to dryness and stored at −20° C.

Analysis of polysome

Polysomes were layered on 15 to 30 % sucrose density gradient made up

with Buffer I and centrifuged in a Beckman SW 50.1 rotar at 40,000 rpm for 60 min at 1° C. After the gradient was fractionated and monitored, the relative amounts of polysomes, monosomes and subunits were calculated in the same manner as described by SARUYAMA *et al.* (1980)

Assay of amino acid incorporation

The reaction mixture (50 μl) consisted of 100 mM Tris-HCl, pH 7.8, 60 mM NH_4Cl, 3.5 mM magnesium acetate, 6 mM 2-mercaptoethanol, 1 mM adenosine triphosphate (ATP), 0.2 mM guanosine triphosphate (GTP), 5 mM creatine phosphate, 1.5 μg creatine kinase, 5 μCi [^{14}C]amino acid mixture, 20 μM each of cold 20 amino acids, and one of the following protein-synthesizing systems: (a) crude S-30 fraction (50 μg protein), (b) polysomes (3 A_{260} units) plus supernatant fraction (50 μg protein), and (c) ribosome (3 A_{260} units) plus supernatant fraction (50 μg protein) plus R17 RNA (20 μg). Incorporation of [^{14}C]amino acids into proteins was determined as reported previously (OSHIMA *et al.*, 1980).

Protein determination

Protein concentration was determined by the method of LOWRY *et al.* (1951). Bovine serum albumin was used as standard.

Chemicals

ATP, GTP, creatine phosphate, creatine kinase were obtained from Boehringer Mannheim. Aurintricarboxylic acid (ATA) was obtained from Wako Pure Chemical Industries, Ltd. L-[^{14}C(U)]-Amino acid mixture containing 15 amino acids (112-536 μCi/nmol each) was purchased from the New England Nuclear Corp., Boston, Mass.

Results

Endogenous protein-synthesizing systems

Crude S-30 extracts prepared from *Vibrio* ABE-1 and *P. aeruginosa* were tested for incorporation of amino acid into proteins at various temperatures between 0° C and 30° C in the absence of exogenous mRNA. The relative activity of protein synthesis at the temperatures below 25° C was much higher in *Vibrio* ABE-1 than in *P. aeruginosa* but the net synthesis in *Vibrio* ABE-1 was less than that in *P. aeruginosa* above 15° C (Fig. 1). As seen in this figure, these results show that the relative rates of protein synthesis with endogenous mRNA at various temperatures in the bacteria were similar to those with poly(U) (OSHIMA *et al.*, 1980).

Purified polysome-directed protein-synthesizing systems

It was reported in *E. coli* that low-temperature-induced block of the initiation of translation caused accumulation of monosomes and subunits

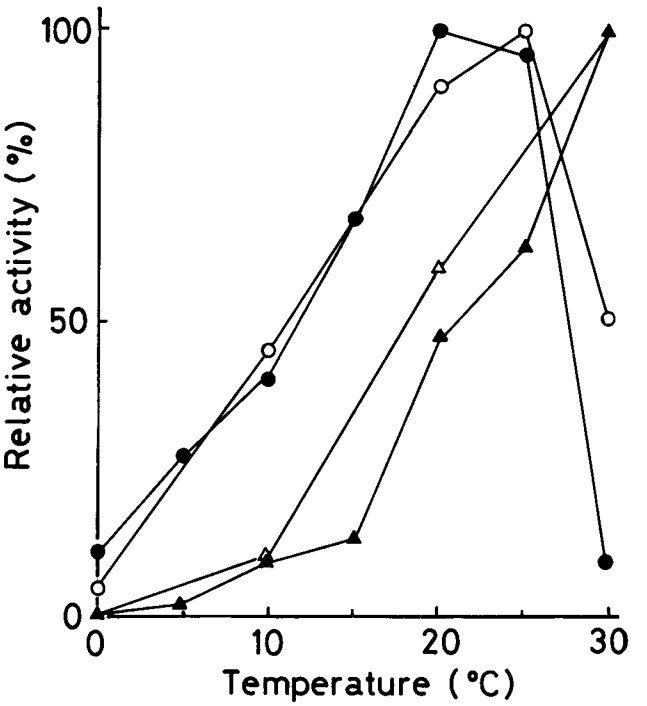

Fig. 1. Relative activities of incorporation of [^{14}C]amino acids into proteins by crude S-30 systems of *P. aeruginosa* (▲) and *V.* ABE-1 (●) at various temperatures. The initial rates of incorporation were determined from the slope in the linear portion of the reaction at each temperature. The maximum values in this experiment were 126.5 cpm/min (*P. aeruginosa*) and 32.6 cpm/min (*V.* ABE-1). Poly (U)-directed incorporations of [^{14}C]phenylalanine into polyphenylalanine by *P. aeruginosa* (△) and *V.* ABE-1 (○) were cited from a previous paper (OSHIMA *et al.*, 1980).

(SZER, 1970; DAS and GOLDSTEIN, 1968; FRIEDMAN *et al.*, 1969, 1971), but in *Pseudomonas fluorescens*, a psychrotroph, such the accumulation could not be observed at 5° C or below (BROEZE *et al.*, 1978). Thus, we attempted to examine the effect of temperature on the initiation activity of polysomes of *P. aeruginosa* and *Vibrio* ABE-1. The protein-synthesizing activities of the polysome systems were measured at 25° C (a little higher than the maximum temperature for growth of *Vibrio* ABE-1), and at 10° C (near the minimum

temperature for growth of *P. aeruginosa*), in the presence or absence of ATA which is known to be an inhibitor for initiation of translation (TAI *et al.*, 1973). In the absence of ATA, the rates of protein synthesis in *P. aeruginosa* and *Vibrio* ABE-1 at 10° C were 13 and 41 % of the rates obtained at 25° C under the same assay condition. Temperature-dependence of the protein synthesis with the polysome systems was also similar to those with crude S-30 fractions and the poly(U) systems. In the presence of ATA, the incorporation of amino acid into proteins ceased abruptly after about 8 to 10 min of incubation at 25° C in both bacteria, while the incorporation continued further in the absence of ATA (Fig. 2A). However, the incorporation in the *P. aeruginosa* system at 10° C proceeded in the similar manner whether ATA was added or not (Fig. 2B). Above results suggest a possibility that the activity of reinitiation is repressed by low temperatures as 10° C in *P. aeruginosa*, but

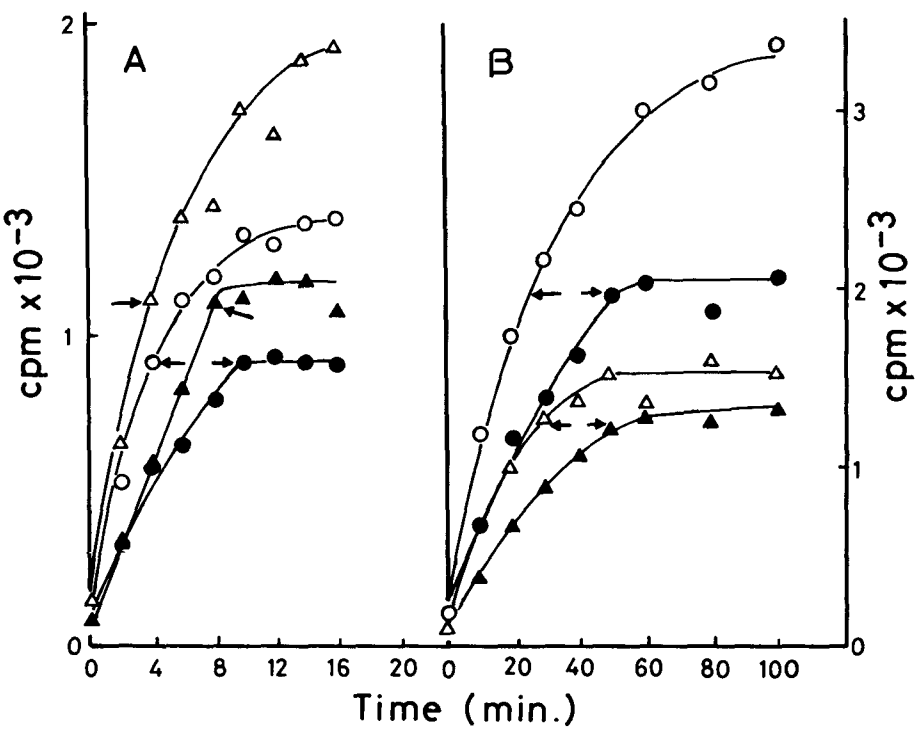

Fig. 2. Purified polysome-directed protein-synthesis. Incorporation of [^{14}C]amino acids was estimated at 25° (A) and 10°C (B) with purified polysome system of *P. aeruginosa* (△,▲) and *V.* ABE-1 (○,●) in the absence (△, ○) or presence (▲, ●) of ATA (200 μM for *P. aeruginosa* and 100 μM for *V.* ABE-1). Supernatant fraction of *V.* ABE-1 was used in this experiment. Symbol (←), see the legend to Table 1.

not in *Vibrio* ABE-1. Therefore, we examined the composition of ribosomes under the same conditions as indicated in Fig. 2A, B. As shown in Table 1, the polysome content in *P. aeruginosa* system was markedly reduced during the incubation with ATA or at 10° C, but the content in *Vibrio* ABE-1 system incubated at 10° C was as much as that at 25° C if ATA was not added.

Table 1. Effect of ATA on the composition of ribosomal fraction

Treatment	Incubation temperature	Relative amount of fractions (%)					
		V. ABE-1			*P. aeruginosa*		
		S	M	P	S	M	P
0 Time		9	19	72	7	30	63
−ATA	25° C	9	33	58	11	43	47
+ATA	25° C	11	40	49	12	62	26
−ATA	10° C	9	37	54	15	61	23
+ATA	10° C	10	55	35	11	65	23

Each sample was drawn at the period indicated as a symbol (←) in Fig. 2. Analytical method of ribosomal fraction is described in Materials and Methods. Reaction conditions were the same as Fig. 2.
S, subunits; M, monosomes; and P, polysomes.

Effect of ribosomes and crude initiation factors on the polysome systems

In order to examine whether the ribosomes and initiation factors of *Vibrio* ABE-1 restore the reduction of the initiation activity in *P. aeruginosa* at 10° C, we studied the effects of these components on the protein synthesis in *P. aeruginosa* polysome system. Neither ribosomes nor crude initiation factors of *Vibrio* ABE-1 had such effect (Fig. 3). The failure of these components to restore the repressed initiation of *P. aeruginosa* at 10° C might be due to the inability to exchange these components between the bacterial systems. In fact, such a specificity was observed when the translation of coli phage R17 was carried out with the hybrid ribosomes of *E. coli* Q13, *P. aeruginosa* and *Vibrio* ABE-1 (Table 2).

Discussion

Relative activities of polypeptide elongation with crude S-30 systems in the present study were very similar to those with poly(U)-directed polyphenylalanine-synthesizing systems at various temperatures (OSHIMA *et al.*, 1980). BROEZE *et al.* (1978) reported that the repression of the elongation rate at low temperature was the same extent both in a mesophile (*E. coli*) and a psychrotroph (*P. fluorescens*), but we found that the repression in a psy-

chrophilic *Vibrio* ABE-1 was much lower than that in a mesophilic *P. aeruginosa* (Fig. 1).

In the purified polysome systems of the two bacteria, at low temperature, almost the same elongation activity was observed whichever supernatant fraction from *P. aeruginosa* or *Vibrio* ABE-1 was used (data not shown). Furthermore the ribosomal 50S subunits of *Vibrio* ABE-1 were reported to contribute to maintenance of high activity in poly(U)-directed polyphenylalanine synthesis at 0° C (OSHIMA *et al.*, 1980). These results suggest that the high elongation rate of polypeptides with the natural mRNA at low temperatures is likely due to the ribosomes of the psychrophile, but not to any constituent of supernatant.

On the other hand, several investigators suggested that initiation steps of translation in mesophilic bacteria are more cold-sensitive than elongation steps, and low temperature causes accumulation of the ribosomal subunits and monosomes (BROEZE *et al.*, 1978; DAS and GOLDSTEIN, 1968; FRIEDMAN *et al.*, 1969, 1971). However, we reported previously that there was no

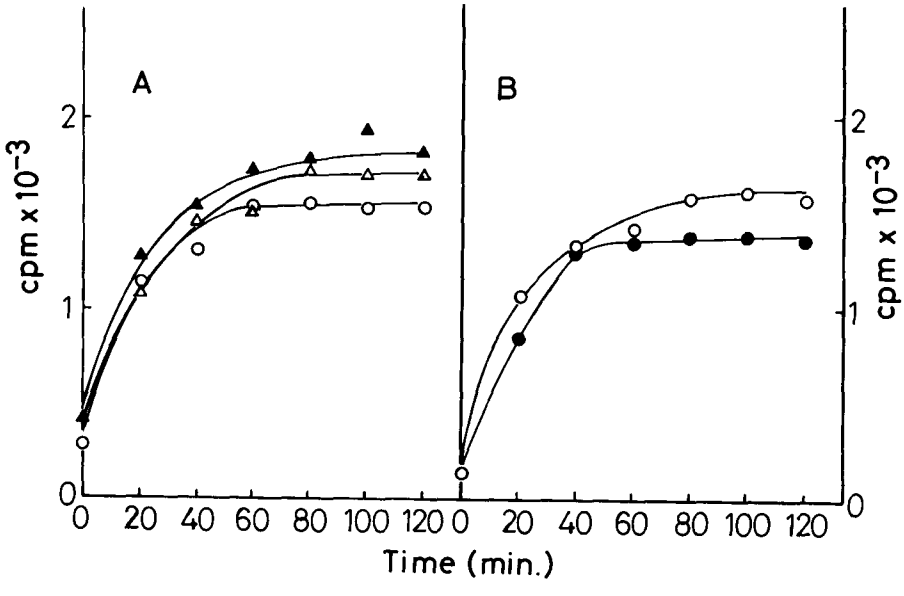

Fig. 3. Effect of ribosomes and crude initiation factors on the *P. aeruginosa* polysome system. All reactions were carried out at 10°C. (A), Addition of ribosomes (final concentration was 3 A_{260} units) to the *P. aeruginosa* polysome system. (▲) *V.* ABE-1 ribosomes; (△) *P. aeruginosa* ribosomes; (○) no ribosome. (B), Addition of crude initiation factors from *V.* ABE-1 (about 75 μg) to the *P. aeruginosa* polysome system. (●) initiation factors; (○) no factor.

significant accumulation of monosomes and subunits in *P. aeruginosa* at 0° C (SARUYAMA *et al.*, 1980). As shown in Fig. 2B, *P. aeruginosa* was able to elongate polypeptides at 10° C, but from the experimental results shown in Fig. 2B and Table 1 we have concluded that the reinitiation step was completely blocked at that temperature. We suggested previously that the elongation-termination steps of translation in whole cells of *P. aeruginosa* might be blocked at 0° C (SARUYAMA *et al.*, 1980), but present results indicate that the initiation step is the most cold-labile one in translation of *in vitro* protein-synthesizing system of the bacteria. On the contrary, the initiation of translation in *Vibrio* ABE-1 is operated actively at low temperature.

The failure of the crude initiation factors or the ribosomes from *Vibrio* ABE-1 to relieve the cold-sensitivity of *P. aeruginosa* polysome system (Fig. 3A, B) may be closely related to the specificity of these components between *Vibrio* ABE-1 and *P. aeruginosa*. LEFFLER and SZER (1973) reported that such a specificity exists in the binding of the ribosomes to the mRNA(s). The translation with R17 RNA in *P. aeruginosa* or *Vibrio* ABE-1 ribosome system was greatly enhanced by addition of the ribosomal 30S subunits from *E. coli*

Table 2. Effect of *E. coli* ribosomal subunits on translation of R17 RNA

Ribosomal fraction	Addition (*E. coli* subunit)	Protein synthesis (cpm/40 min)
E. coli	——	3029
P. aeruginosa	——	658
P. aeruginosa	30S	2301
P. aeruginosa	50S	735
V. ABE-1	——	613
V. ABE-1	30S	2410
V. ABE-1	50S	569
————	30S	325
————	50S	231
————	——	212

Basal protein-synthesizing system used in this experiment was (c) system described in Materials and Methods. Small subunits (1 A_{260} unit) or large subunits (2 A_{260} units) of *E. coli* were added to the system consisting of a desired ribosomal fraction and *E. coli* supernatant fraction. After incubation at 30° C for 40 min, radio-activity in hot TCA-insoluble fraction was assayed.

(Table 2). The results indicate that the specificity is mainly due to the ribosomal 30S subunits.

Since the initiation factors from different bacteria were reported to be interchangeable with each other (LEFFLER and SZER, 1973; SZER and BRENOWITZ, 1970), the results shown in Fig. 3B indicate that the initiation factors of *Vibrio* ABE-1 do not have any positive role in reinitiation process under low temperatures.

If some suitable natural mRNA would have been found, we could more concretely elucidate what is the real factor which participates in the active protein synthesis at low temperature. Accordingly it may be the only thing to say at present that ribosomal 30S subunits of psychrophilic bacteria may have an important role in the initiation step of translation, especially in binding of ribosomes to mRNA at low temperature.

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