The effect of temperature shifts on protein synthesis by the psychrophilic bacterium *Vibrio* sp. strain ANT-300

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In the psychrophilic bacterium *Vibrio* sp. strain ANT-300, the temperature-related characteristics of protein synthesis in cells grown at 0 °C differed from those of cells grown at 13 °C. Cells grown at 0 °C and 13 °C transported amino acids at the same rates, dependent on the temperature at which rates were measured. The rates of protein synthesis in extracts of cells grown at 0 °C and at 13 °C differed, as a result of the changes in the properties of the soluble fraction involved in protein synthesis. Concurrently, levels of more than 24 polypeptides in the soluble fraction changed considerably. These results suggest that the difference in temperature dependence of protein synthesis in cells grown at various temperatures may be brought about by specific changes in the levels of a small number of polypeptides (less than 15 % of the total number of proteins detected by silver-staining) in response to a change in temperature.

Introduction

It is well known that the relationships between temperature and the growth rate of bacteria are characterized by straight lines in the Arrhenius profiles in the middle temperature range, with distinct changes in slope at temperatures higher and lower than the optimum range. The forms of such Arrhenius curves are quite similar among various species and strains of bacteria that possess the capacity to grow at a range of different temperatures (Mohr & Krawiec, 1980). There are no significant correlations between the values of temperature characteristics representing the Arrhenius constant (μ_A) of the exponential growth rates in the cells of psychrophiles, psychrotrophs and mesophiles (Ratkowsky *et al.*, 1982, 1983; Reichardt & Morita, 1982).

Given the myriad of chemical reactions involved in growth, it would seem likely that the relationship between temperature and metabolic control must be quite complex. It is unclear how cells regulate their metabolic reactions so as to maximize their growth rates in the middle temperature range, over which their temperature characteristics remain approximately constant. It is also unclear how such optimization is prevented at temperatures outside this range. Several previous studies have found that one or more reactions become rate-limiting at extreme temperatures, either

high (Patterson & Gillespie, 1972) or low (Broeze *et al.*, 1978; Das & Goldstein, 1968; Inniss & Ingraham, 1978), as a result of the inability of cells to compensate for thermally induced changes in the conformation of proteins.

With the advent of methods for measuring the amounts of individual proteins, it has been shown that metabolic coordination in *Escherichia coli* within the middle temperature range seems to be controlled predominantly by modulation of enzymic activities rather than by modulation of the levels of individual proteins, and that restricted growth outside this range is accompanied by marked changes in the levels of a relatively small number of proteins (Herendeen *et al.*, 1979; Lemaux *et al.*, 1978). After a shift-up in temperature, transient changes in the rates of synthesis of a small number of proteins appear to be induced by the promotion of transcription that is regulated by an alternative sigma subunit of RNA polymerase (σ^{32}), the product of the *rpoH* (*htrR*) gene, a process that is known as the heat-shock response (Cowing *et al.*, 1985; Grossman *et al.*, 1984; Neidhardt & VanBogelen, 1981; Taylor *et al.*, 1984; Yamamori *et al.*, 1978; Yamamori & Yura, 1980, 1982; Yura *et al.*, 1984). Furthermore, more than a dozen proteins, none of which are heat-shock proteins, have been shown to be induced by a shift-down in temperature (Goldstein *et al.*, 1990; Jones *et al.*, 1987).

It has been also shown that, in psychrotrophic and psychrophilic bacteria, a shift-up in temperature induces

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Abbreviation: AIB, α -aminoisobutyric acid.

increases in the rates of synthesis of heat-shock proteins (McCallum *et al.*, 1986). However, there are no studies, to our knowledge, of the effects of temperature on the levels of individual proteins in psychrotrophic and psychrophilic bacteria. In order to clarify the way in which psychrophilic bacteria regulate their metabolism so that they can grow efficiently at extremely low temperatures, the effects of temperature on the rates of protein synthesis *in vivo* and *in vitro*, and also on the levels of individual proteins in *Vibrio* sp. strain ANT-300 cells, were examined. The results reported here indicate that a temperature characteristic representing the activation energy of protein synthesis *in vivo* and *in vitro* in cells grown at 0 °C differed from those of cells grown at 13 °C, and that the levels of more than 24 polypeptides in the soluble fraction changed considerably, depending on the growth temperature.

Methods

Bacterial strain and medium. *Vibrio* sp. strain ANT-300 was obtained from Dr R. Y. Morita (Oregon State University, USA). Cells were grown in a complex medium (pH 7.8) that contained 1.2 g yeast extract, 2.3 g trypticase peptone, 0.3 g sodium citrate, 0.3 g potassium glutamate, 50 mg NaNO₃ and 5 mg FeSO₄ per litre of artificial seawater, the latter consisting of 2.8% (w/v) Jamarine-S in distilled water, as described by Geesey & Morita (1975).

Preparation of cell-free extracts. Cells were grown to the late-exponential phase at either 0 °C or 13 °C, harvested by centrifugation at 10000 g for 10 min at 0 °C and washed with artificial seawater supplemented with 50 mM KCl, 10 mM-magnesium acetate, 1 mM dithiothreitol, 10 mM-Tris/HCl (pH 7.6). Cells were ruptured with a French press (Ohtake, Japan), and crude S30 extracts were prepared by the method of Nirenberg (1963). These extracts were fractionated into a supernatant (S100 fraction) and a pellet by centrifugation at 105000 g for 120 min. The pellets were resuspended in the initial volume of the supplemented seawater by gentle homogenization and centrifuged again at 105000 g for 120 min. After the removal of the supernatants, washed pellets (R100 fraction) were resuspended in the initial volume of the supplemented seawater.

Assays of protein synthesis *in vivo* and *in vitro*. Cells were grown to the mid-exponential phase of growth at either 0 °C or 13 °C. Portions of each culture (5 ml) were mixed with 2 vols of medium, pre-equilibrated to the desired temperature, and held at this temperature. [¹⁴C]Leucine (12.9 GBq mmol⁻¹) was added to a final concentration of 74 KBq ml⁻¹, and 0.5 ml portions of culture were removed at appropriate intervals and mixed with 5 ml cold 10% (w/v) trichloroacetic acid (TCA). The precipitated samples were filtered through membrane filters (0.45 µm; Toyo Roshi Kaisha, Japan) and washed with 10 ml cold 10% TCA; and then the filters were put into scintillation vials which contained 0.3 ml ethyleneglycol monoethylether. Aquasol-2 (5 ml) was added to each vial, and the radioactivity was counted in an LKB RackBeta liquid scintillation counter.

For protein synthesis *in vitro*, the reaction mixture consisted of 60 mM-NH₄Cl, 20 mM-KCl, 8 mM-magnesium acetate, 1 mM-ATP, 20 µM-GTP, 5 mM-potassium phosphoenolpyruvate, 30 µg of pyruvate kinase ml⁻¹, 50 mM-Tris/HCl (pH 7.6) and 74 KBq ml⁻¹ of a mixture of

¹⁴C-labelled amino acids. To initiate the reaction, S30 extracts (0.5 ml; about 30 mg protein ml⁻¹) were added to the above reaction mixture (1.0 ml), which was pre-equilibrated to the appropriate test temperature. At regular intervals, aliquots (0.2 ml) were removed and put into tubes containing 2 ml cold 10% TCA; then samples were treated for counting of radioactivity as described above.

The rates of protein synthesis *in vivo* and *in vitro* were calculated from the amounts of amino acids incorporated into the TCA-insoluble fraction during the course of incubations for 60 min and 10 min, respectively.

Transport of amino acids into whole cells. Cells grown to an optical density of about 0.2 units at 600 nm at either 0 °C or 13 °C were harvested and resuspended to give 1/50 of the original culture volume in the artificial seawater (pH 7.8). Portions of the suspension (1.0 ml) were mixed with 4.0 ml of artificial seawater pre-equilibrated to the desired temperature, and then either [¹⁴C]leucine (12.9 GBq mmol⁻¹) or α-aminoiso[¹⁴C]butyrate (2.26 GBq mmol⁻¹) was added to a final concentration of 18.5 KBq ml⁻¹. At appropriate intervals, aliquots (0.2 ml) were passed over prewashed membrane filters which were then washed three times with 2 ml of artificial seawater. After washing, filters were put into scintillation vials and treated for counting of radioactivity as described above. Other samples were immersed in 5 ml portions of cold 10% TCA and then separated into TCA-soluble and TCA-insoluble fractions by centrifugation at 3000 g for 10 min. The TCA-insoluble fraction was resuspended in the artificial seawater and passed over prewashed membrane filters which were then washed three times with 5 ml cold 10% TCA. These fractions were treated for counting of radioactivity as described above.

Analysis of proteins by two-dimensional gel electrophoresis. Cells grown to the mid-exponential phase at 0 °C were immediately labelled with [¹⁴C]leucine (12.9 GBq mmol⁻¹, 74 KBq ml⁻¹) for 60 min at 0 °C or for 30 min at 13 °C. Other portions were transferred to a temperature of 13 °C and aliquots of culture were removed after a further 3, 6 and 30 h and then labelled for 30 min at 13 °C. Cells labelled with [¹⁴C]leucine were collected by centrifugation, washed with the artificial seawater and then resuspended in 10 mM-Tris/HCl buffer (pH 7.4) that contained 5 mM-MgCl₂, 10 mM-PMSF, and 50 µg pancreatic RNAase ml⁻¹. The cells were lysed by sonication and then lysates were treated with 50 µg DNAase ml⁻¹ at 0 °C for 15 min. Solid urea was added to a final concentration of 9 M-urea. After the urea had dissolved completely at room temperature, an equal volume of lysis buffer, which contained 9 M-urea, 2% (v/v) Nonidet P-40, 1.6% (v/v) Ampholine (pH 5–7), 0.4% Ampholine (pH 3–10) and 5% (v/v) mercaptoethanol, was added.

Two-dimensional gel electrophoresis was done by the method of O'Farrell (1975). Slab gels were stained with 'Daiichi', silver stain, impregnated with Enlightning as an enhancer and dried for autoradiography. Fluorographs were obtained by exposing Fuji X-ray films to the treated slab gels.

Nomenclature for polypeptides. Cellular proteins were separated by isoelectric focusing in one dimension ('horizontal') and by electrophoresis in the presence of SDS in the second dimension ('vertical'). The individual polypeptides that were detected by silver staining were numbered from the upper left-hand corner of the gel horizontally to the right-hand corner. The numbering of polypeptides was continued from the upper line of polypeptides to the lowest line in the same manner, ending in the bottom right-hand corner.

Chemicals. Yeast extract and Trypticase peptone were obtained from Difco and BBL, respectively. Jamarine-S was purchased from Jamarine Laboratories. (Osaka, Japan). L-[U-¹⁴C]Leucine, α-amino-[1-¹⁴C]isobutyric acid and the U-¹⁴C labelled amino acid mixture were obtained from Amersham. Aquasol-2 and Enlightning were obtained from New England Nuclear. Ampholines were obtained from LKB.

Fig. 1. Effects of temperature on growth of cells of *Vibrio* sp. strain ANT-300. A culture incubated at either 0 °C (a) or 13 °C (b) was divided into three parts and the various portions were incubated at 13 °C (○), 7 °C (⊕) or 0 °C (●) from time zero.

Nonidet P-40 was purchased from BHL Chemicals. Acrylamide, methylene-bisacrylamide and tetramethylene diamine were purchased from Eastman-Kodak. 'Daiichi' silver stain was purchased from Daiichi Pure Chemicals. (Tokyo, Japan). Pancreatic RNAase, DNAase and PMSF were purchased from Sigma.

Results

Growth and protein synthesis after a shift in temperature

A shift-up in temperature from 0 °C to either 7 °C or 13 °C resulted in an increased rate of growth of *Vibrio* sp. strain ANT-300, as measured by the optical density of cultures at 600 nm (Fig. 1). An initial stimulation of growth was followed by a period of several hours during which the rates gradually increased until a final steady-state growth rate, characteristic of the new growth temperature, was established. The final phase is not shown in Fig. 1(a), but the steady-state growth rate at 13 °C (doubling time 5.5 h) was the same as that of cells grown at 13 °C (Fig. 1b). After a shift-down in temperature from 13 °C to 0 °C or 7 °C, the growth rate of cells was immediately reduced. After this, there was a very slow and gradual increase in growth rate. An eventual steady-state rate, characteristic of incubation at 0 °C (doubling time 11 h) was established almost 33 h after the shift-down to 0 °C.

The effects of temperature on protein synthesis by cells grown at 0 °C and 13 °C are shown in Fig. 2. The rates of protein synthesis increased with the increase in temperature, but showed a different temperature dependence. In cells grown at 0 °C, protein synthesis at 7 °C and 13 °C continued at a rate equal to 1.7 and 2.3 times, respectively, that in cells incubated at 0 °C. The rate of protein synthesis during incubation at 13 °C of cells grown at 13 °C was approximately 1.3 times higher than

Fig. 2. Effects of temperature on rates of protein synthesis in cells of *Vibrio* sp. strain ANT-300 grown at 0 °C (a) and 13 °C (b). Results are means from three independent experiments. ○, Incubation temperature 13 °C; ⊕, 7 °C; ●, 0 °C.

that of cells grown at 0 °C, but the rate of protein synthesis in cells grown at 13 °C was very significantly reduced by incubation at either 7 °C or 0 °C. The rate of protein synthesis during incubation at 0 °C was reduced to approximately 20% of the value during incubation at 13 °C, as compared to the 50% reduction in the case of cells grown at 0 °C.

Temperature dependence of amino acid transport

To investigate whether the discrepancy in temperature-dependent protein synthesis resulted from a restricted response of substrate transport to various temperatures, the effects of temperature on the accumulation of amino acids in cells grown at 0 °C and in cells grown at 13 °C were examined. Table 1 compares the temperature-dependent uptake of α -aminoisobutyrate (AIB) by cells grown at 0 °C and by cells grown at 13 °C. Both groups of cells accumulated [¹⁴C]AIB at a constant rate for at least 5 min, after which the rates of accumulation decreased gradually. The rate of accumulation of AIB increased with increasing temperature of incubation. The ratios of the initial rates of accumulation at 7 °C and 13 °C to that at 0 °C were 2.19 and 5.22, respectively, for cells grown at 0 °C, and 2.24 and 5.28, respectively, for cells grown at 13 °C. The relative response to higher temperatures was the same in both groups of cells. All of the radioactive materials accumulated in whole cells could be extracted

Table 1. Temperature dependence of [^{14}C]AIB transport by cells grown at 0 °C and 13 °C

The initial rates were estimated from tangents drawn to uptake curves at zero time. Results in this Table are means of values from three independent experiments.

Sample	Temp. (°C)	$10^{-3} \times [^{14}\text{C}]\text{AIB accumulation}$ [d.p.m. (mg cells) $^{-1}$]					Ratio of initial rates
		2 min	5 min	10 min	15 min	30 min	
Cells grown at 0 °C	0	1.1	2.7	5.2	7.3	13.2	1.00
	7	2.4	5.9	12.1	17.5	31.9	2.19
	13	5.6	14.1	27.3	32.7	42.0	5.22
Cells grown at 13 °C	0	1.0	2.5	4.3	6.4	12.5	1.00
	7	2.2	5.6	12.2	17.5	31.1	2.24
	13	5.3	13.2	22.7	30.6	42.5	5.28

Fig. 3. Effects of temperature on incorporation of [^{14}C]leucine. Cells grown at either 0 °C (a) or 13 °C (b) were labelled with [^{14}C]leucine at 13 °C (○), 7 °C (◻) or 0 °C (●). [^{14}C]Leucine incorporation was measured in whole cells (---) and in a TCA-soluble fraction (—).

by treating the cells with either 10% TCA or water at 100 °C for 15 min. The accumulated AIB had the same mobility during thin-layer chromatography as pure AIB. These results suggest that the accumulated AIB is free in the cytoplasm and is in an unchanged form. By contrast,

leucine was accumulated first in the cytoplasm and then it was incorporated into cellular components, in particular into proteins. Fig. 3 shows the effects of temperature on the uptake of [^{14}C]leucine in the total-cell and TCA-soluble fractions from cells grown at 0 °C and from cells grown at 13 °C. The accumulation of ^{14}C -leucine in the two fractions from both groups of cells continued at a constant rate for at least 3 min and then lower rates were recorded. The initial rate of accumulation in the total-cell and TCA-soluble fractions of cells grown at 0 °C responded to different temperatures of incubation in a manner similar to that of cells grown at 13 °C. In the TCA-soluble fraction of both groups of cells, the level of accumulated leucine reached a maximum after 10 min at 13 °C, and after about 30 min at 7 °C and 0 °C, and then decreased gradually. The accumulation of leucine in the total-cell fraction of both groups of cells showed a different response to incubation temperatures as time passed.

Effects of temperature on protein synthesis by cell-free extracts

The kinetics and extent of incorporation of ^{14}C -labelled amino acids by crude S30 extracts prepared from cells grown at 0 °C and cells grown at 13 °C are shown in Fig. 4. The activities of S30 extracts from cells grown at 13 °C were usually 3 to 5 times higher than those of cells grown at 0 °C, although the activities varied from preparation to preparation. At 0 °C, S30 extracts from cells grown at 13 °C incorporated amino acids into the TCA-insoluble fraction at approximately 29 to 33% of the rate at 13 °C, whereas S30 extracts from cells grown at 0 °C synthesized proteins at 0 °C at approximately 45 to 50% of the rate at 13 °C. The effects of temperature on the rates of protein synthesis by whole cells grown at 0 °C and at 13 °C and the rates of protein synthesis *in vitro* by S30

Fig. 4. Effects of temperature on protein synthesis *in vitro*. Crude S30 extracts were prepared from cells grown at either 13 °C (○) or 0 °C (●); the extracts were then incubated with a mixture of ¹⁴C-labelled amino acids at 13 °C (—) or 0 °C (---). Data obtained from a single preparation are plotted. Although there was an approximately 30% difference in the absolute values obtained from various preparations, the same temperature dependence was observed in all experiments.

extracts from both groups of cells were compared (Fig. 5). The patterns of temperature dependence of the rates of protein synthesis *in vivo* and *in vitro* observed with cells grown at 0 °C were different from those observed with cells grown at 13 °C, and there was a somewhat higher relative decrease in the rate of protein synthesis in whole cells grown at 13 °C.

The fractions designated S100 and R100 were prepared separately from crude S30 extracts of cells grown at 0 °C and at 13 °C. They were then combined, and the

Fig. 5. Temperature dependence of protein synthesis *in vivo* and *in vitro*. Cells were grown at 13 °C (○) and at 0 °C (●). The rates of protein synthesis in whole cells (a) and in S30 extracts (b) at the temperatures indicated are shown.

capacities of the combined fractions to synthesize proteins at 0 °C and 13 °C were determined (Table 2). Compared with the activity of S30 extracts from cells grown at 13 °C, the activities of a mixture of the S100 fraction from cells grown at 13 °C with washed ribosomes from cells grown at either 13 °C or 0 °C decreased by approximately 20% and 40% respectively, but the ratios of activities at 0 °C to those at 13 °C did not change. By contrast, in S30 extracts from cells grown at 0 °C the ratios of activities at 0 °C to those at 13 °C

Table 2. Temperature-dependent protein synthesis in combinations of soluble fractions and ribosomes prepared from cells grown at 0 °C and 13 °C

Values in parentheses indicate the temperature at which cells were grown before preparation of the S30 and S100 fractions and the washed ribosomes (R100). Reaction mixtures were set up as described in Methods using 1.14 mg protein of an S30 fraction ml⁻¹, 0.48 mg protein of an S100 fraction ml⁻¹ and 0.66 mg protein of an R100 fraction ml⁻¹, as indicated. Data represent radioactivity incorporated into the TCA-insoluble fraction over a period of 10 min. Results in this Table are means of values from three independent experiments.

Fractions present	10 ⁻³ × ¹⁴ C-labelled amino acid incorporation (d.p.m. ml ⁻¹)		Ratio of activities at 0 °C and 13 °C
	0 °C	13 °C	
S30 (13 °C)	1.53	5.21	0.294
S100 (13 °C) + R100 (13 °C)	1.24	4.34	0.286
S100 (13 °C) + R100 (0 °C)	0.86	3.15	0.273
S30 (0 °C)	0.55	1.14	0.482
S100 (0 °C) + R100 (13 °C)	0.78	1.57	0.497
S100 (0 °C) + R100 (0 °C)	0.45	0.89	0.506

Fig. 6. Proteins in S100 fractions after fractionation by two-dimensional gel electrophoresis. An S100 fraction was prepared from cells grown at 0 °C (a) and 13 °C (b). The gels were stained with silver, and characteristic polypeptides in each sample numbered as described in Methods.

Fig. 7 (facing page). Effects of a shift-up in temperature on protein synthesis in cells of *Vibrio* sp. strain ANT-300 grown at 0 °C. Cells were labelled with [^{14} C]leucine for 60 min at 0 °C (a) or for 30 min at 13 °C after 0 h (b), 3 h (c), 6 h (d), and 30 h (e) post-shift. The polypeptides characteristic of cells grown at 0 °C and 13 °C are indicated by circles and boxes, respectively. In panels (b)–(e), the numbers indicate polypeptides whose rates of synthesis were stimulated by a shift-up in temperature.

differed from that of S30 extracts from cells grown at 13 °C. The ratio of activities at 0 °C to those at 13 °C of a mixture of the S100 fraction from cells grown at 0 °C with washed ribosomes from cells grown at either 0 °C or 13 °C was almost the same as that of S30 extracts from cells grown at 0 °C. In both cases, the ratios of activities at 0 °C to those at 13 °C were largely dependent on the characteristics of the S100 fractions, and not on the source of the washed ribosomal fractions. The activities in a mixture of the S100 fraction and washed ribosomes from cells grown at 0 °C decreased to approximately 80% of the pre-fractionation values at 0 °C and 13 °C. By contrast, the activities in a mixture in which the S100 fraction from cells grown at 0 °C was combined with washed ribosomes from cells grown at 13 °C were 40% greater than the activities of S30 extracts from cells grown at 0 °C.

Analysis of proteins by two-dimensional gel electrophoresis

The electrophoretograms of proteins in S100 fractions prepared from cells grown at either 0 °C or 13 °C are shown in Fig. 6. The pattern of the polypeptides from the S100 fraction of cells grown at 0 °C differed qualitatively from that from cells grown at 13 °C. The levels of 28 polypeptides (numbered in Fig. 6*a*) were very high in cells grown at 0 °C, while the levels of 25 polypeptides (numbered in Fig. 6*b*) were very high in cells grown at 13 °C. The number of polypeptides that were variable in terms of level was less than 15% of the total number of polypeptides (256) detected by silver-staining of electrophoretograms. To examine whether the synthesis of each polypeptide differed in temperature dependence, cells grown at 0 °C were labelled with [¹⁴C]leucine for 60 min at 0 °C or for 30 min at 13 °C at various times after the shift-up to 13 °C, and then radioactive polypeptides were analysed by autoradiography. Equal amounts of protein from each sample were subjected to two-dimensional gel electrophoresis and the resulting autoradiograms are shown in Fig. 7.

The major polypeptides in cells grown at 0 °C were preferentially synthesized during incubation at 0 °C, as indicated by circles in Fig. 7(*a*). After a shift-up in temperature the synthesis of most polypeptides was immediately stimulated and the increases in the rates of synthesis of 18 polypeptides (numbered in Fig. 7*b*) were especially marked. Two of these polypeptides, indicated by boxes (nos 53 and 121), were characteristic of cells grown at 13 °C. The other 16 polypeptides (nos 40, 64, 70, 71, 96, 119, 122, 134, 143, 156, 165, 170, 171, 189, 198 and 206) were characteristic of cells grown at 0 °C. The rates of synthesis of these polypeptides changed with time after the shift-up to 13 °C. Of 16 polypeptides, the rates

of synthesis of eight (nos 119, 134, 143, 156, 165, 189, 198 and 206) were reduced after 3 h post-shift (Fig. 7*c*), while the remaining eight polypeptides (nos 40, 53, 64, 70, 71, 96, 121 and 170) continued to be synthesized until 6 h post-shift (Fig. 7*d*). After 30 h post-shift, two polypeptides (nos 53 and 121) continued to be synthesized in large amounts; the rates of synthesis of an additional 10 polypeptides, all of which are characteristic of cells grown at 13 °C (nos 29, 39, 46, 47, 66, 95, 109, 111, 117 and 137) also increased markedly (Fig. 7*e*).

Discussion

From the results of the present study, it appears that the protein synthesis activity at a low temperature is sufficient to permit growth at lower temperatures, and that this is a result of changes in the properties of soluble components involved in protein synthesis. Changes in the membrane transport of amino acids do not appear to be involved. Furthermore, the levels of a small number of proteins in the soluble fraction change after a shift in temperature. It is, therefore, suggested that the difference in the temperature dependence of protein synthesis of cells grown at different temperatures may be related to the changes in the levels of this small number of proteins. Considering that the number of proteins detected by silver-staining of two-dimensional gels is probably less than 10% of the total number of proteins in the cell, the possibility should not be overlooked that the proteins, which play a regulatory role in the response to a temperature shift, might exist as minor proteins undetectable by silver-staining.

With a shift-up in temperature from 0 °C to 13 °C, the rate of total protein synthesis increased gradually to a new steady-state level, which was established after a period of a few generations. The rates of synthesis of individual polypeptides changed with time (Fig. 7), exhibiting apparently complex kinetics. At first, the increases in the rates of synthesis of at least 18 polypeptides were very marked (Fig. 7*b*) and, after 30 h post-shift, considerable increases in the rates of synthesis of a dozen polypeptides, each characteristic of steady-state growth at 13 °C, were noted (Fig. 7*e*). These effects on rates of synthesis of certain polypeptides cannot be explained only in terms of general changes in the rate of protein synthesis as it responds passively to temperature, or by the direct effects of restrictions in the uptake of amino acids. In fact, transport of leucine and AIB into cells grown at either 0 °C or 13 °C responded to temperature in a similar manner (Table 1 and Fig. 3). Therefore, it is possible that the specific effects seen in the rates of synthesis of some polypeptides are an active regulatory response.

When cells of a typical mesophilic bacterium, *E. coli*, are transferred from a low to a high temperature, the rates of synthesis of a small number of proteins, the heat-shock proteins, increase transiently (Yamamori *et al.*, 1978; Yamamori & Yura, 1980, 1982). The high-temperature regulon of *E. coli* consists of 17 genes that are transiently induced, with high rates of expression, by a shift-up in temperature (Neidhardt *et al.*, 1983). These genes are subject to their own individual controls, but their coordinated response to temperature is regulated by an alternative sigma factor of RNA polymerase (σ^{32}), the product of the *rpoH* (*htrR*) gene (Grossman *et al.*, 1984; Taylor *et al.*, 1984; Yura *et al.*, 1984). Such induction of heat-shock proteins seems to represent, in part, a homeostatic response at the cellular level to environmental changes in both eukaryotes and prokaryotes. Moreover, such a response system may well be conserved in psychrotrophs and psychrophiles.

In the psychrophilic bacterium *Aquaspirillum* sp. Res-10, the synthesis of at least 10 proteins (detected by one-dimensional gel electrophoresis) is induced by a shift-up in temperature from 0 °C to either 15 °C or 20 °C, with maximal induction after 4 h. Furthermore, induction of these proteins seems to be regulated at the transcriptional level (McCallum *et al.*, 1986).

In contrast to the response to heat shock, little is known about the effects of a shift-down in temperature on protein synthesis. In *E. coli*, about two dozen proteins are preferentially synthesized after a down-shift, in spite of the reduction in the rate of synthesis of most cellular proteins. Among these proteins, seven have been identified; some are involved in transcription and translation (Jones *et al.*, 1987).

In the psychrophilic bacterium *Vibrio* sp. strain ANT-300, changes in the rates of synthesis of individual polypeptides may also be associated with an active regulatory response at the transcriptional and/or translational level. With respect to the nature of signals responsible for an active response, a variety of evidence (Gallant *et al.*, 1977; Lee *et al.*, 1983a, b; Little *et al.*, 1983; Little & Bremer, 1984; Pingoud *et al.*, 1983; Reynolds *et al.*, 1983; Ryals *et al.*, 1982a, b; Williams *et al.*, 1983) suggests that adenylylated nucleotides, such as ppGpp and AppppA which accumulate as a result of amino acid restriction, nutritional shift, temperature shift and oxidative stress, might be involved in the preferential synthesis and/or regulation of RNA.

In cells of *Vibrio* sp. strain ANT-300, a shift-up in temperature caused an increased accumulation of ppGpp within a maximum at 4 h and a simultaneous decrease in the synthesis of RNA. By contrast, a shift-down in temperature caused an immediate decrease in RNA synthesis and a concurrent decrease in levels of ppGpp (unpublished data).

In *E. coli*, cells exposed to any one of various stresses exhibit different patterns with respect to the accumulation of adenylylated nucleotides (VanBogelen *et al.*, 1987). Moreover, VanBogelen & Neidhardt (1990) have shown that ppGpp is neither sufficient nor necessary for signal induction of the heat-shock response. Thus, the exact mechanisms by which mediators exert their regulatory effects are still unknown in most cases.

Finally, the results presented here suggest that the adaptation in the rates of synthesis of certain proteins in the psychrophilic bacterium *Vibrio* sp. ANT-300 enable it to grow efficiently at 13 °C and 0 °C. The levels of a small number of proteins only have to be adjusted with the change in temperature. Such a temperature-induced control of protein synthesis may be related to a general adaptive response of both eukaryotes and prokaryotes to shifts in temperature in the natural environment.

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