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Altered heat-shock response in polyamine-depleted bacteria

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A polyamine-auxotrophic mutant of *E. coli* was cultivated in the presence or absence of putrescine and submitted to heat shock over 3 different ranges of temperature. In all cases, protein synthetic capacity measured in comparison to that of cultures at the preshift temperature was much higher in polyamine-depleted bacteria under thermic stress. Addition of putrescine only before the shift-up was able to restore gradually normal control of the relative protein synthetic capacity.

Heat shock *Polyamine* *Putrescine* (Escherichia coli) *Protein synthesis*

1. INTRODUCTION

When growing bacterial cells are submitted to a sudden increase in incubation temperature there is a rapid change in the synthesis of several proteins. This phenomenon, known as the heat-shock response, also occurs in eucaryotes and archaebacteria and involves some highly conserved proteins [1,2]. So far 17 polypeptides with a transiently accelerated rate of synthesis have been described in *E. coli* [1]. The functions and mechanism of induction of these proteins have not yet been completely elucidated.

On the other hand, polyamines have been implicated in the biosynthesis of DNA, RNA and proteins and in the regulation of transcription and translation [3]. Since the heat-shock response is apparently controlled at the level of transcription [4], we decided to investigate the participation of polyamines in bacterial thermic stress. We report here that when cultures of a polyamine-auxotrophic mutant growing in the absence of putrescine are transferred to a higher incubation temperature, there is a transient marked increase in the protein synthetic capacity in comparison to the activity at the preshift temperature. Addition of putrescine to polyamine-starved cultures restores the profile of relative synthetic activity characteristic of supplemented bacteria only when supplied before the shift-up. The degree of recovery of the pattern depends on the extent of time in the presence of putrescine.

Dedicated to Dr Luis F. Leloir on the occasion of his 80th birthday

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2. MATERIALS AND METHODS

E. coli BGA8, a mutant unable to synthesize putrescine, has been described [5]; MA 17, the parental strain with normal polyamine metabolism, was kindly provided by Dr W.K. Maas. Bacteria were grown in Davis and Mingioli minimal medium [6] supplemented with thiamine (5 μg/ml), L-leucine and L-threonine (50 μg/ml each) and glucose (0.4%); they were starved of polyamine as in [5].

To measure protein synthetic capacity under heat shock, polyamine-depleted or -supplemented exponential cultures at a given temperature were shifted to the new temperature indicated in each case. Aliquots of 10 μl removed at different moments were labeled for 2 min with [^{35}S]methionine (New England Nuclear, 0.5 μM,

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25 μCi/ml). The pulse was terminated by addition of 25 μg/ml methionine and the samples chilled immediately. After precipitation with trichloroacetic acid and filtration, radioactivity was measured in a liquid scintillation counter. The results are expressed relative to those of cells growing at the preshift temperature, pulse-labeled at the moment of change.

For analysis of the synthesized proteins, 0.4 ml cell culture was pulse-labeled as described above, except that 50 μCi/ml [^{35}S]methionine was used. The cell extracts were subjected to 10% polyacrylamide gel electrophoresis in the presence of SDS, according to Laemmli and Favre [7], using an acrylamide/*N,N'*-methylenebisacrylamide ratio of 100:1. The gels were dried and exposed to an X-ray film at −70°C.

3. RESULTS AND DISCUSSION

3.1. *Effect of temperature shift-up on growth and relative protein synthetic capacity in polyamine-supplemented or -starved bacteria*

An increase in growth temperature evokes changes in the polypeptides synthesized, both qualitatively and quantitatively [1,4]. The heat-shock response in *E. coli* occurs over a broad range of temperatures. We have chosen 3 different ranges of change in temperature to study the effect of polyamine content during thermic stress: 28–42, 37–50 and 28–50°C.

During the shift from 28 to 42°C (Δ 14°C), which leads to an increased growth rate (fig.1A,B), there was an enhancement of relative protein synthetic activity with and without putrescine, fol-

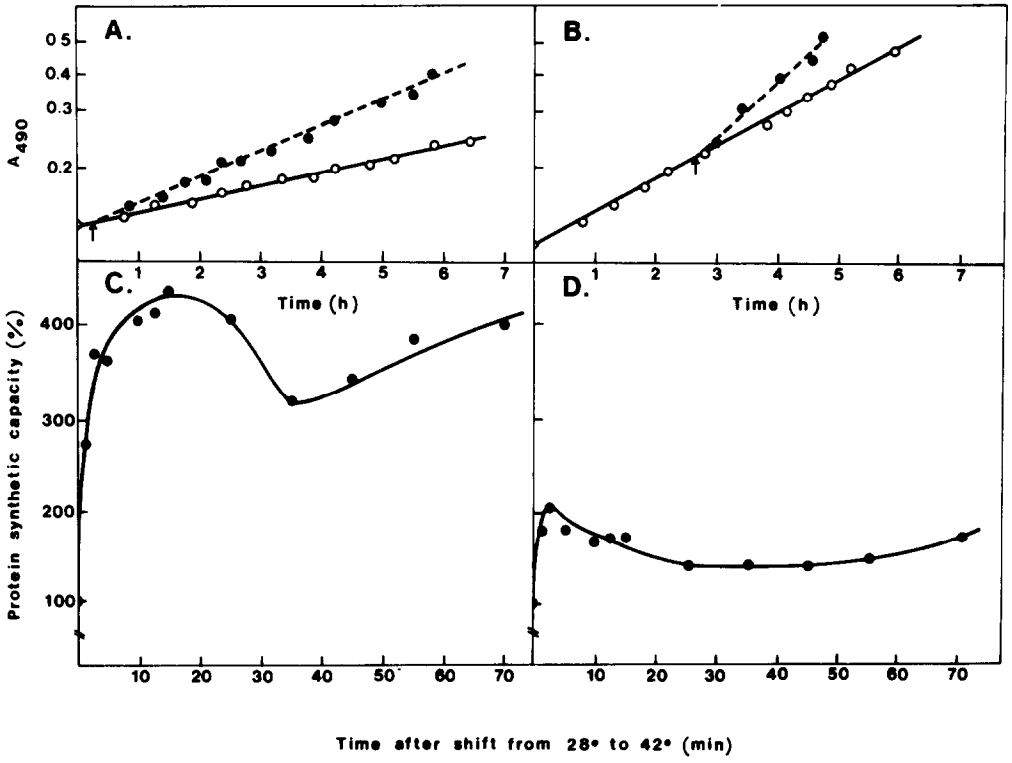

Fig.1. Effect of changing the incubation temperature from 28 to 42°C in the absence or presence of putrescine. Cultures of polyamine-starved (A) and unstarved (B) cells growing exponentially at 28°C were divided into two aliquots at the moment indicated by the arrow. Growth was continued further at 28 (○) and 42°C (●); it was monitored by measuring the absorbance at 490 nm. Relative protein synthetic capacity in the absence (C) and presence (D) of putrescine was estimated as indicated in section 2. Values were normalised for culture absorbance. Radioactivity incorporated into trichloroacetic acid-precipitable material at 28°C, taken as 100%, was 2338 and 7701 cpm for C and D, respectively.

lowed by a slight decrease and a new increase in protein synthesis (fig.1C,D). This kinetics is in agreement with results obtained by others on the rate of bulk protein synthesis [8]. Moreover, the changes were more pronounced in the absence of putrescine, in both the extent of activation and duration of the peak.

A similar range of temperature shift from 37 to 50°C (Δ 13°C), which is a transition from an optimal to a lethal temperature, resulted in growth interruption (fig.2A,B). There was again a peak of increased activity in the absence of putrescine, but for a shorter period and with lower intensity, and a steady decrease in synthetic capacity afterwards. In the presence of putrescine only faint activation was observed, followed immediately by an abrupt decline in protein synthetic rate (fig.2C,D).

Finally, a larger difference in incubation temperature, from 28 to 50°C (Δ 22°C), extending from a sub-optimal to a lethal temperature, led again to a halt in growth (fig.3A,B). In this case there was only a small increase of relative protein synthesis in the absence of putrescine and a steady decline afterwards, while in cultures supplemented with polyamine a sharp drop in activity occurred from the start (fig.3C,D).

In different cultures of polyamine-depleted bacteria there may be a small temporal variation in the appearance of the increase in relative activity. The magnitude and duration of the peak of relative protein synthetic capacity depended on the temperatures before and after the shift (figs 1–3). Notwithstanding, cells growing in the absence of putrescine displayed a much larger synthetic rate relative to that at the preshift temperature in all cases, reflecting an ability to over-react to heat shock when compared to their low preshift synthesis. The relative synthetic capacity of polyamine-supplemented bacteria was consistently smaller at similar periods after the change, sug-

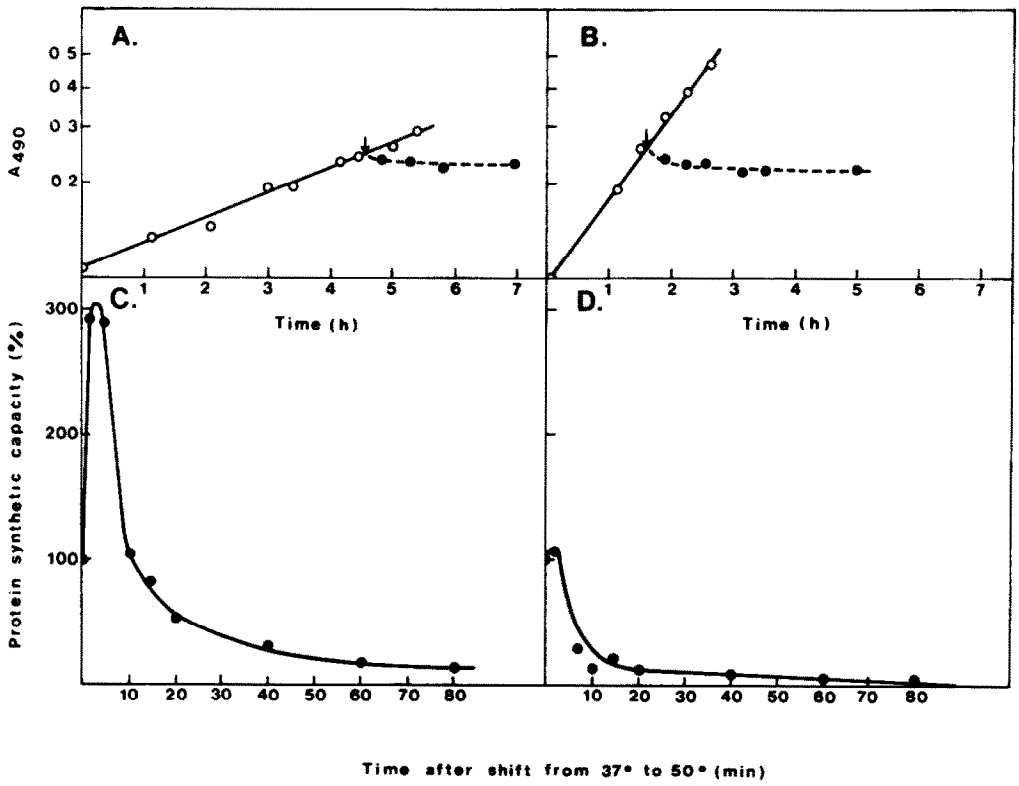

Fig.2. Effect of changing the growth temperature from 37 to 50°C in polyamine-depleted or -supplemented cultures. Experimental details as for fig.1. Growth at: (○) 37°C, (●) 50°C. Radioactivity incorporated at 37°C (100%) was 5971 and 33723 cpm for C and D, respectively.

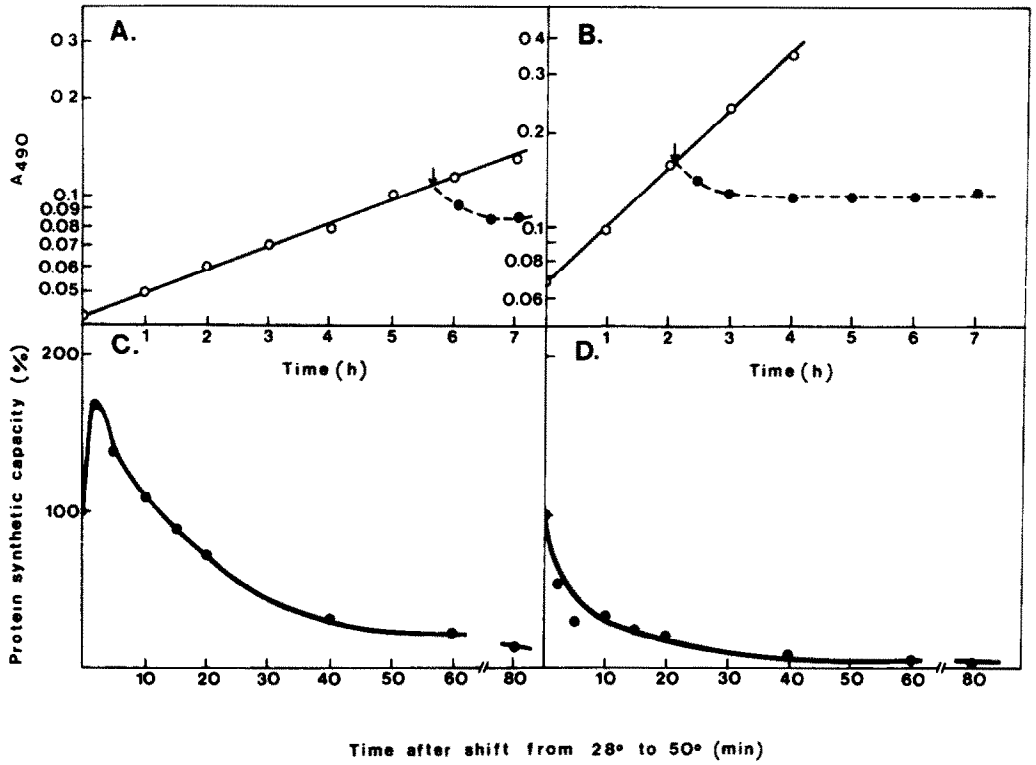

Fig.3. Effect of changing the incubation temperature from 28 to 50°C in polyamine-unsupplemented or -supplemented cells. Experimental details as for fig.1. Growth at: (○) 28°C, (●) 50°C. Radioactivity incorporated at 28°C (100%) was 3649 and 15212 cpm for C and D, respectively.

gesting that the polycation evokes a more controlled reaction to the stress.

To exclude the possibility that the altered response in polyamine-depleted bacteria could be attributed to a slower growth rate, control experiments were performed in which putrescine-supplemented cells were cultivated in the presence of 0.2% acetate instead of glucose as carbon source. Under these conditions cell growth rate decreased about 50%, reaching values similar to those of polyamine-depleted cultures. When acetate substituted for glucose there was no effect on the putrescine-supplemented pattern of relative synthetic capacity. Moreover, *E. coli* MA 17, the parental strain of *E. coli* BGA8 with normal polyamine content, behaved similarly to cultures cultivated with putrescine (not shown).

3.2. *Polypeptide patterns of polyamine-starved and -unstarved cells under heat shock*

Fig.4 shows the patterns of radioactive polypeptides obtained when cells growing exponentially at 28°C were transferred to 42°C and pulse-labeled for 2 min with [^{35}S]methionine at different moments after the shift-up. One-dimensional gel electrophoresis revealed no major qualitative differences between putrescine-depleted and -supplemented bacteria. However, in the latter some bands appeared earlier, such as the 72 and 83 kDa peptides. In addition, other peptides could be detected longer after the heat shock: the 47 kDa polypeptide began to fade gradually after 5 min in extracts of unsupplemented cells, but had about the same intensity all the time in bacteria with putrescine. Similar behavior could be observed in the 40 kDa peptide. The fact that these proteins continued to be expressed for a longer period after the shift-up in the presence of polyamines could indicate a different control in the biosynthesis and/or functioning of the corresponding mRNAs. When the cells were transferred from 28 to 50°C or 37 to 50°C, temporal differences were also

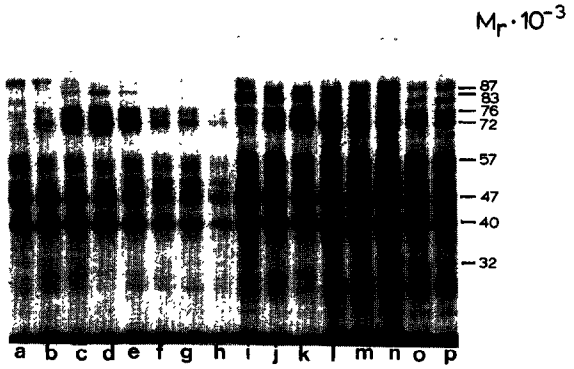

Fig.4. SDS-polyacrylamide gel electrophoresis of labeled polypeptides synthesized in the absence or presence of putrescine during a heat shock from 28 to 42°C. Aliquots of cell cultures growing without (a–h) and with (i–p) putrescine were prepared for electrophoresis as described in section 2. Cultures at 28°C (a,i) were shifted up to 42°C and labeled after 2 (b,j), 5 (c,k), 10 (d,l), 20 (e,m), 40 (f,n), 60 (g,o), and 80 min (h,p). Equal amounts of radioactivity were applied to each lane.

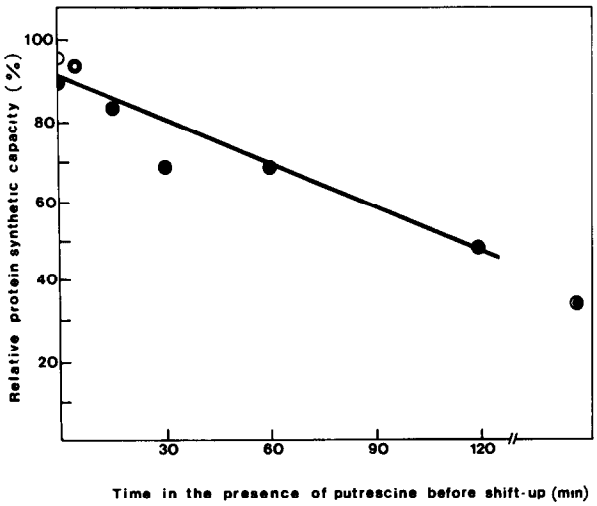

Fig.5. Effect of putrescine addition to polyamine-starved cultures shifted from 28 to 50°C. Aliquots of polyamine-depleted cell cultures growing at 28°C were supplied with putrescine at different moments before (●) or 5 min after (⊙) the change to 50°C. Relative protein synthetic capacity was estimated 10 min after the transfer to 50°C as described in section 2. Values corresponding to cultures grown throughout without (○) or with (◉) putrescine were included as controls.

detected between polyamine-depleted and -supplemented cells. The increased protein synthesis observed after the stress in these cases seemed to be mainly due to the induction of heat-shock peptides (not shown).

3.3. *Effect of putrescine addition to polyamine-depleted cultures*

Polyamine-starved bacteria submitted to an increase in growth temperature, such as from 28 to 50°C, failed to show the rapid fall in protein synthetic capacity characteristic of putrescine-supplemented cultures, as depicted in fig.3. When the polycation was supplied to polyamine-depleted cells growing at 28°C, no effect could be detected in the restoration of the sharp drop in relative protein synthetic activity if the addition was at the moment of the shift to 50°C or later. Nevertheless, there was a recovery of the pattern of activity observed in polyamine-supplemented bacteria if putrescine was added before the shift-up (fig.5). The effect was larger with increasing time of pre-incubation with the polyamine indicating that the restoration of the control by putrescine is a slow process.

E. coli BGA8, the strain used in these studies, has been shown to respond abnormally when subjected to stresses such as amino acid deprivation [9] or the addition of aminoglycoside antibiotics [5]. The present results show that after heat shock there is an unexpected enhancement of the relative protein synthetic capacity in polyamine-depleted cells. This increase can be restricted on addition of putrescine before the temperature shift-up. Although more work is necessary to understand the mechanism of this alteration, our results suggest for the first time the involvement of polyamines in the control of the heat-shock response in bacteria.

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