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Synthesis of unique proteins at the onset of carbon starvation in *Escherichia coli*

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SUMMARY

Escherichia coli bulk protein synthesis continued during the first 3–4 h of carbon starvation at 50–75% that of non-starved (growing) cells. Two-dimensional gel electrophoresis analysis of in vivo pulse-labelled proteins resolved at least 30 polypeptides with new or increased synthesis, relative to total protein synthesis, during this time. Among these polypeptides were several that were also synthesized by ethanol-treated *E. coli* (heat-shock proteins). In addition, a number of unique polypeptides were synthesized by carbon-starved cells. These 'starvation proteins' may be involved in survival of the starving bacteria.

INTRODUCTION

Nongrowing bacterial cells are of considerable applied interest: in antibiotic production; in achieving separation of growth and production phases of bacteria used in industrial fermentation processes, allowing for a more efficient conversion of raw material to product and obviating the problem of reversion of genetically engineered organisms; in immobilized cell bioreactors, where the limited containment volume requires stable nongrowing microbial cells; and as inocula for seeding desired environments. In addition, starvation is often experienced by bacteria in their natural habitat, making studies on starvation survival of ecological importance [8,13].

We have been studying protein metabolism in such cells, hoping to identify processes that aid in their survival and metabolic stability. Our previous work involving peptidase-deficient mutants of *Escherichia coli* and *Salmonella typhimurium* [13], and inhibition of synthesis of active proteins in wild type *E. coli* by chloramphenicol or amino acid analogues [14], strongly suggests that new proteins are synthesized at the onset of carbon starvation, and that such protein synthesis is beneficial for survival during starvation.

We report here that the relative synthesis of about 30 individual polypeptides increases at the onset of carbon starvation in *E. coli*; several of these polypeptides are not synthesized at a detectable level in glucose-growing bacteria, while others are made in growing cells but their relative abundance among the total proteins synthesized increases dur-

ing the first few hours of starvation. Comparisons of two-dimensional (2-D) gel electrophoresis maps [11] with the published literature as well as direct analysis of ethanol-treated cells indicate that several heat-shock proteins are among those synthesized by carbon-starved *E. coli*.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions

The experimental procedure by which the data of Fig. 1 were obtained was as follows: *E. coli* K-12 wild-type was grown in defined M9 medium containing 0.4% (w/v) glucose at 37°C as described previously [13,14]. A logarithmically growing culture was harvested by centrifugation (3000 × *g*, 15 min), washed, and resuspended in M9 minus glucose to an A_{660} of 0.3 (one A_{660} unit equals about 10^9 cells/ml). The suspension was then divided into four aliquots, each receiving one of the following additions: [A] 0.4% glucose; [B] no additions; [C] 5 μM each of 20 amino acids; [D] 0.4% glucose plus 10% (v/v) ethanol. Temperature was maintained at 37°C throughout. Subculture [A] continued logarithmic growth for at least 2.5 h. The A_{660} of subcultures [B], [C], and [D] did not change during the course of the experiment, indicating lack of growth; thus, cells in these three subcultures were subjected to glucose starvation in the absence of amino acids, glucose starvation in the presence of amino acids, and ethanol shock, respectively. Previous results have established that at the concentration added, the amino acids stimulate bulk protein synthesis and stabilize the starving bacteria, but do not cause cell multiplication (Ref. 13, and A. Bockman, C. Reeve, and A. Matin, unpublished data).

Analysis of protein synthesis

Samples (0.8 ml) of the four subcultures were removed at specified times during the experiment and pulse-labelled with L-[^{35}S]methionine (10^{-8} M; 25 μCi/ml; 1000 Ci/mmol; New England Nuclear, Boston, MA) for 6 min. Labelled proteins were precipitated with trichloroacetic acid (10% w/v), washed, and resolved by 2-D polyacrylamide gel electrophoresis [11]. Conditions for electrophoresis were: first dimension, isoelectric focusing to equilibrium (6000 V/h), 4% acrylamide, 0.4% pH 3/10 plus 1.6% pH 4/7 ampholines (Bio-Rad Laboratories, Richmond, CA), 500000 cpm ^{35}S-labelled polypeptides per tube gel; second dimension, sodium dodecyl sulphate polyacrylamide gel electrophoresis, 10% acrylamide [5]. Newly synthesized polypeptides were visualized by fluorography of dried slab gels [6]. Comparisons were made by superimposing X-ray film (Eastman Kodak, Rochester, NY) fluorographs of slab gels run in parallel.

RESULTS

Polypeptide synthesis in growing, carbon-starved, and ethanol treated E. coli subcultures

Protein synthesis in the logarithmically growing subculture gave a 2-D gel pattern (Fig. 1A) similar to that published by others for *E. coli* [2,4,11,12,16]. The carbon-starved subculture, in addition to synthesizing many of the same polypeptides, also synthesized new ones (Fig. 1B); the synthesis of about 30 polypeptides was either newly initiated or increased relative to total protein synthesis. Some of these polypeptide spots are enclosed in circles in Fig. 1B for illustration. Samples for the 2-D gel maps in Fig. 1 were normalized with respect to total (bulk) protein synthesis; thus, the intensity of a given polypeptide spot is theoretically proportional to its relative abundance among the total translation products.

Among the polypeptides with increased relative rates of synthesis during carbon starvation, a spot with the same approximate charge and size coordinates of the *E. coli groEL* gene product was identified by comparison with published 2-D gel maps [9,11,12]. Since this gene product is among heat-shock proteins known to be induced in *E. coli* upon temperature shift from 37 to 42°C, ethanol treatment, and other stresses [1,2,4,9,10,15], it was of interest to determine what other heat-shock proteins were synthesized during carbon starvation. To do this, we examined the effect of ethanol treatment on the protein synthesis pattern of *E. coli* K-12 in

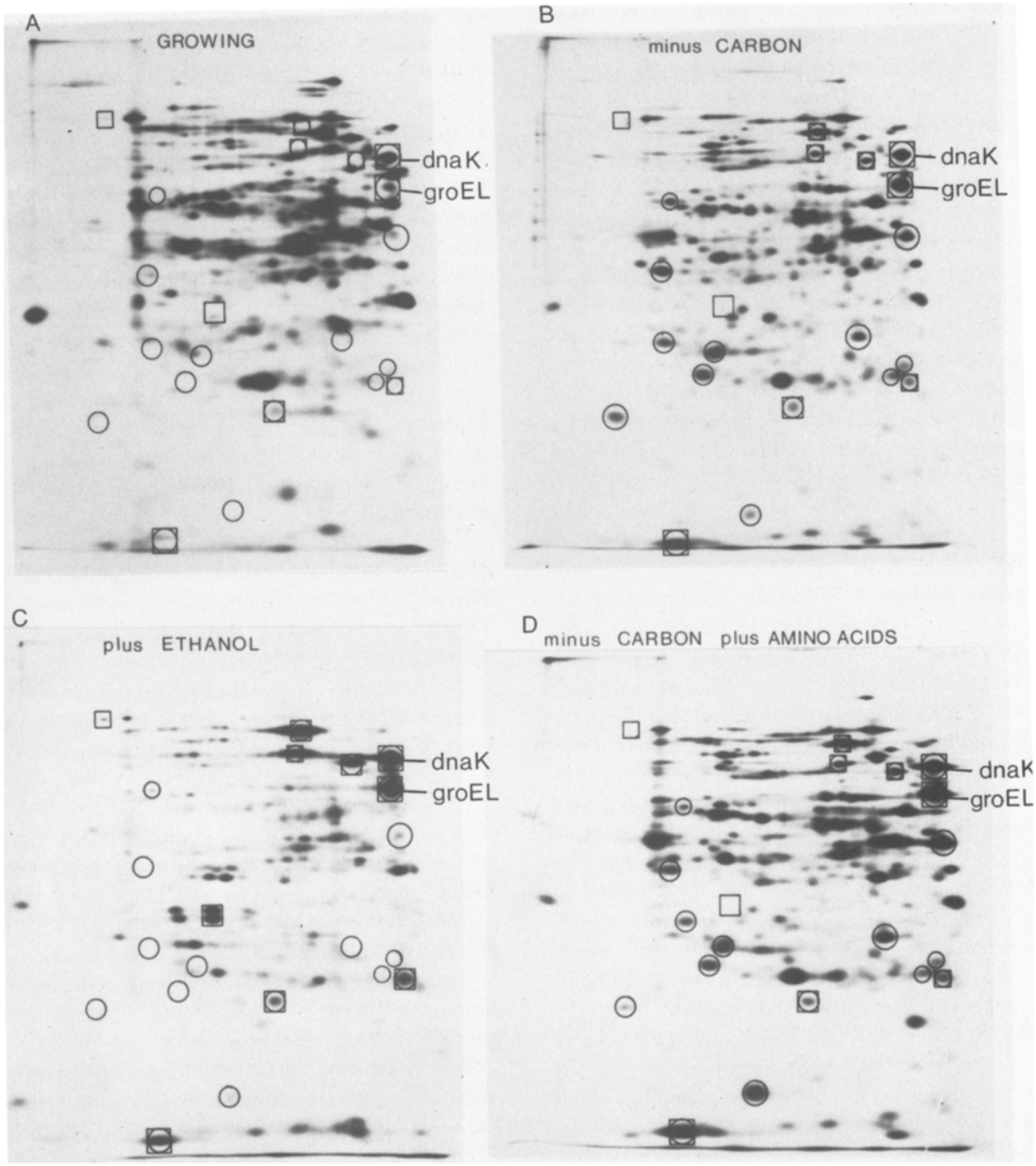

Fig. 1. Two-dimensional polyacrylamide gel electrophoresis maps of protein synthesis by growing, carbon-starved, and ethanol-treated *E. coli* K-12 subcultures. A logarithmically growing culture was divided into four aliquots, each receiving further treatment to obtain the specified conditions. After 30 min of incubation, samples from the four subcultures were pulse-labelled with L-[^{35}S]methionine. Samples for 2-D gels were normalized with respect to total protein synthesis. See Materials and Methods for further details. Panel A, Logarithmically growing subculture (M9 plus 0.4% glucose); panel B, Carbon-starving subculture (M9 minus carbon source; panel C, Ethanol-treated subculture (M9 plus 0.4% glucose plus 10% ethanol); panel D, Carbon-starving subculture with amino acids (M9 minus glucose plus 5μM each of 20 amino acids).Circles illustrate polypeptides induced in carbon-starved cells; squares illustrate polypeptides induced in ethanol-treated cells. The *dnaK* and *groEL* gene product spots are indicated by arrows for reference.

our experimental system. As noted above, ethanol elicits the synthesis of a subset of the heat-shock protein. In agreement with previous reports [10,15], synthesis of many growth-related polypeptides ceased in the ethanol-treated subculture but that of several heat-shock proteins increased, including spots corresponding to the *dnaK* and *groEL* gene products (Fig. 1C). It is evident from a comparison of Fig. 1B and C that, in addition to the *groEL* gene product, synthesis of a number of other heat-shock proteins is also common to glucose-starved and ethanol-treated bacteria. In order to distinguish polypeptides synthesized only by carbon-starved cells from those synthesized both by carbon-starved and by ethanol-treated cells, some polypeptide spots are enclosed in circles and circles within squares, respectively, in Fig. 1.

Effect of addition of amino acids on polypeptide synthesis during carbon starvation

Other workers have recently demonstrated that some *E. coli* heat-shock proteins are synthesized during the *relA*-mediated stringent response to amino acid starvation [10,3b]. Glucose starvation could conceivably create amino acid scarcity in the cells, raising the possibility that the synthesis of new proteins during glucose starvation was mediated by amino acid starvation. We therefore investigated the pattern of protein synthesis by carbon-starving bacteria to which low levels of amino acids (see Materials and Methods) were added. In agreement with previous results [13], bulk protein synthesis rate increased by 20–30% compared to carbon-starved cells not supplemented with amino acids. However, the same new polypeptides were synthesized by carbon-starved *E. coli*, either in the absence or in the presence of added amino acids (Fig. 1B and D, respectively). It therefore is unlikely that amino acid scarcity was directly responsible for the change in protein synthesis patterns observed during glucose starvation.

DISCUSSION

The data of Fig. 1 were obtained using a common cell suspension that was divided into four aliquots, each of which experienced different subsequent conditions, i.e., logarithmic growth, starvation with or without amino acids, or ethanol treatment (see Materials and Methods). Since the temperature was kept constant at 37°C and cell density was kept low, it is unlikely that centrifugation or other experimental manipulations produced the observed changes in cellular protein synthesis patterns. Even if such effects occurred they should have been common to all aliquots of the master suspension. The different protein synthesis patterns found following subsequent different treatments of individual aliquots (growth, starvation, etc.) are therefore regarded to be due to the individual treatments per se. In recent work (J. Schultz and A. Matin, unpublished data) we have obtained starvation conditions by allowing exhaustion of glucose from the culture, obviating the need for centrifugation, etc. to initiate starvation. Under these conditions also, synthesis of the same starvation proteins shown in Fig. 1B and D occurs at the onset of glucose starvation.

We previously demonstrated that inhibition of protein synthesis during carbon starvation, especially in the early phases, greatly compromised survival of the starving bacteria [14]. We now show synthesis of unique proteins in carbon-starved bacteria during the first hour of starvation. Elucidation of the functions of these proteins awaits further studies, but taken together our findings strongly suggest that some of these proteins play a role in survival of the starving bacteria. The *E. coli* response to carbon starvation may be a cellular differentiation to a resting state, comparable to sporulation in some other bacteria.

It is noteworthy that several heat-shock proteins were among those synthesized during carbon starvation. These proteins are synthesized in *E. coli* in response to several different stresses [1,2,4,10,15], and common signal molecules might mediate their expression. *relA*-mediated guanosine tetraphosphate (ppGpp) accumulation in amino acid-starved *E. coli* has been proposed as a factor affecting heat-shock protein synthesis [3b]. Since ppGpp also accumulates during glucose starvation [7], this nu-

cleotide might also affect protein synthesis during carbon/energy starvation.

Research underway is aimed at examining the kinetics of synthesis and exploring the functions of individual starvation proteins. Such studies are likely to suggest ways for enhancing longevity and metabolic productivity of nongrowing bacteria.

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