GENE EXPRESSION IN STRINGENT AND RELAXED STRAINS OF ESCHERICHIA COLI DURING AMINO ACID DEPRIVATION

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Received May 2,1977

SUMMARY

The effect of leucine deprivation on newly synthesized proteins in Escherichia coli was studied by SDS-polyacrylamide gel analysis. Leucine deprivation was found to induce the synthesis of several proteins in a stringent but not in the isogenic relaxed strain of E. coli.

The synthesis of stable RNA in Escherichia coli is greatly reduced by the deprivation of a required amino acid. The control mechanism underlying this response is called the stringent response and depends on the function of the rel gene (1,2). A large body of evidence indicates that the stringent response reflects the regulatory effects of the nucleotide ppGpp, which is formed in rel^+ (stringent) strains in response to amino acid deprivation (for reviews see 3 and 4). Another cellular adjustment related to the stringent response is the regulation of intracellular protein breakdown during amino acid deprivation. Sussman and Gilvarg (5) have shown that nitrogen-source deprivation accelerates protein breakdown in rel^+ but not in isogenic relaxed (rel) strains of E. coli. Furthermore, Goldberg has shown (6) that protein breakdown in bacteria is stimulated by a variety of experimental conditions which curtail stable RNA synthesis. The rel gene product may therefore play a role in both stringent control of RNA synthesis as well as in protein breakdown during amino acid de privation. The mechanism by which the rel^+ gene product regulates protein breakdown is not known. A clue is provided by the observations that the stringent control of protein breakdown is blocked by inhibitors of protein and RNA synthesis (5-8). Possibl protein synthesis is required for the stringent control of protein breakdown (5,8,9). Isogenic rel^+ and rel^- strains, however, show grossly similar rates of residual proteir

ISSN 0006-291X

synthesis during amino acid deprivation (10,11). The effect of the rel A mutation on labeled proteins in amino acid starved cells has been investigated by others (12,13,14 The results are unsatisfactory due to the long labelling time employed (13,14) which tends to reflect the combined effects of protein synthesis and decay. The analytical methods used in these reports are also not sensitive enough to detect subtle changes in protein patterns. We therefore examined more critically the pattern of newly synthesized proteins in response to amino acid deprivation in a pair of isogenic rel^+ and rel strains of E. coli. The analysis was carried out by SDS-polyacrylamide slab-g electrophoretic assays of the newly synthesized proteins labeled with a short pulse of ^{35}S-methionine.

MATERIALS AND METHODS

E. coli NF536 (leu^-, $valS^{ts}$ rel^+) and NF537 (leu^-, $valS^{ts}$, rel A) were grown at 30° with shaking in medium M9 (15) supplemented with 75 µg ℓ-luecine/ml. Cells were harvested at 2 x 10^8/ml by filtration using 25 mm Uni-pore polycarbonate membrane filters (0.2 µm size, Bio-Rad Laboratories). Collected cells were resuspended in leucine-free medium at 2 x 10^8/ml and incubated immediately at 30° with shaking. Filtration through membrane filters provided a fast and reproducible means of harvesti and washing cells. The total procedure took less than 5 min. Proteins were pulse-labeled for 3 min. with 2.5 µCi/ml of ^{35}S-methionine (200 Ci/mmole, New England Nuclea before and after leucine deprivation. Pulse-labelling was terminated by adding 2 ml of 2% casamino acid solution containing 20% glycerol to 1 ml of labeled cells. Cells were pelleted by centrifugation and solubilized in SDS as described by Laemmli (16). Portions of the lysate were precipitated and washed with 5% trichloroacetic acid (TCA) on Gilman glass-fiber filters followed by ethanol washing in preparation for scintillation counting. RNA was labeled by adding ^{3}H-uridine at 0.5 µCi/ml (37.6 Ci/mmole, New England Nuclear) immediately after leucine deprivation. Samples of 100 µl each were withdrawn at different times and precipitated with 5% TCA. The TCA-precipitates were filtered on glass-fiber filters and washed with 5% TCA followed by 95% ethanol. The dried filters were counted in a scintillation spectrometer using a toluene-based counting mixture. The electrophoretic separation of proteins on 10% polyacrylamide

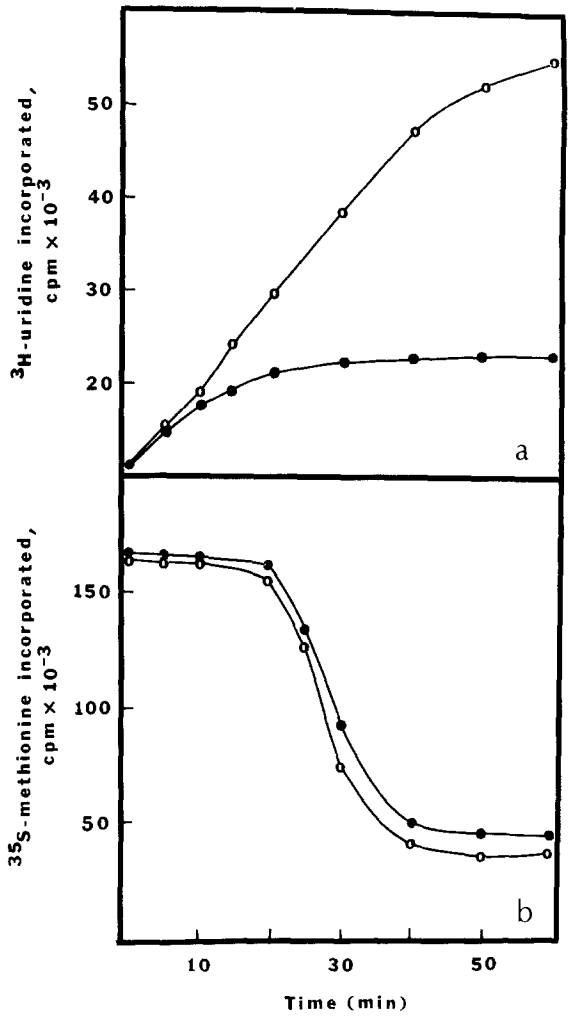

Figure 1. ^{3}H-uridine and ^{35}S-methionine incorporation in NF536 and NF537 during leucine deprivation. Cells grown in leucine-supplemented medium was filtered, washed and resuspended in leucine-free medium as described. Zero time in both figures indicates the time when medium change was completed.
(a) ^{3}H-uridine incorporation. 0.5 µCi ^{3}H-uridine was added to 1 ml leucine-free culture at zero time. Samples of 100 µl each were removed for counting at the time indicated.
(b) ^{35}S-methionine incorporation. Leucine-free cultures were pulse-labeled with 2.5 µCi ^{35}S-methionine/ml for 3 min. at the time indicated. Samples of 100 µl each were used for radioactivity counting. NF536, (●); NF537 (○).

slab-gel was according to the method described by O'Farrell et al. (17). The buffer system of Tris-glycine at pH 8.8 was that of Laemmli (16). Autoradiography was made by exposing the dried gels to Kodak No-screen medical x-ray films.

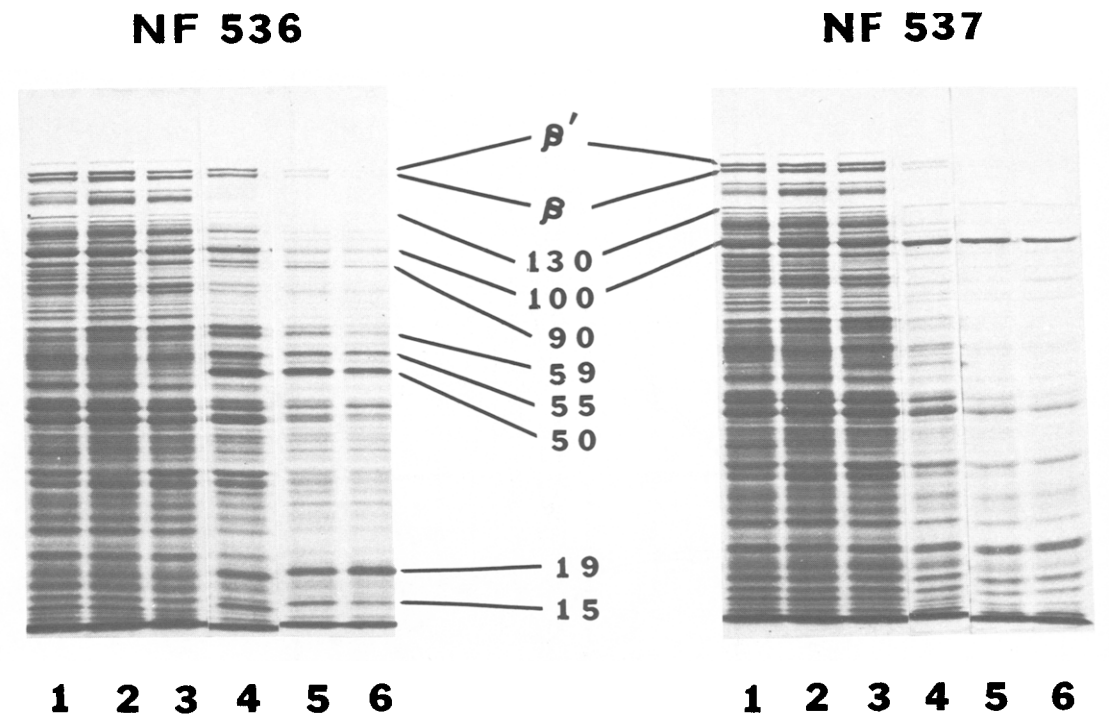

Figure 2. Pulse-labeled protein patterns of NF536 and NF537 during leucine deprivation. Portions of ^{35}S-methionine pulse-labeled samples from Fig. 1b were lysed in SDS and electrophoresed on 10% SDS-polyacrylamide slab-gels. Autoradiograms were made by exposing the dried gels to X-ray films. Each sample shown here represents 3.5 x 10^7 cells. 1, unstarved cells. 2, 10 min. 3, 20 min. 4, 30 min. 5, 40 min. 6, 60 min. after leucine deprivation.

RESULTS AND DISCUSSION

The results of ^{3}H-uridine and ^{35}S-methionine incorporation in the isogenic strai NF536 and NF537 during leucine deprivation are shown in Figure 1. A 15 min. lag wa observed between leucine withdrawal and the onset of the stringent control of RNA synthesis in NF536 (Fig. 1a). This lag is presumably due to the time required for the cells to exhaust the accumulated intracellular leucine. In the isogenic relaxe strain, RNA accumulation proceeded despite the removal of leucine. The reductions in rates of protein synthesis (Fig. 1b) caused by leucine deprivation in both strai were grossly similar, in agreement with the results of others (10,11). An entirely different picture emerged when the pulse-labeled protein patterns were analyzed. T

results of the SDS-polyacrylamide gel separation of the pulse-labeled proteins before and during leucine deprivation are shown in Figure 2. Leucine deprivation caused a general reduction in most pulse-labeled peptides in both the rel$^+$ and rel strains (Fig. 2). The reduction, particularly in the high molecular range, is more rapid in the rel$^-$ strain. One such example is shown by the labelling patterns of the β and β′ subunits of the RNA polymerase (Fig. 2). The synthetic rate of the β and β′ subunits did not change in either strains during the first 20 min. but declined thereafter with halflives of 9 min. 20 sec. and 4 min. 40 sec. in rel$^+$ and rel A strains respectiv The quantitative estimates are based on data obtained from densitometric scanning of the autoradiograms (data not shown). The synthetic rate of low molecular weight pepti (i.e. 20 kilodaltons and less), however, declined more rapidly in the rel$^+$ strain but more slowly in the rel A strain (Fig. 2). Two exceptions are the 19 K (19 kilodalton) and the 15 K peptides which persisted in the starved rel$^+$ strain. The rapid decline in the synthesis of low molecular weight peptides shown by the rel$^+$ strain is expected since some of the low molecular weight peptides are presumably ribosomal proteins (13,14) and that the expression of ribosomal protein genes is under the control of the rel locus (18). Our results, however, do not show any overproduction of low molecular weight peptides by the starved rel A strain, a finding which is contrary to some earlier reports (13,14). The overproduction of low molecular weight peptides has been attributed to an excessive accumulation of truncated peptides (14). The results shown in Figure 2 indicate that the low molecular weight peptides synthesized by the rel A strain during leucine deprivation, by and large, do not appear to be truncated peptides. This is evidenced by the discreteness of the bands (Fig. 2) indicating that these bands are not a random assortment of peptides of different length. A total smeared gel pattern resulted when we either incubated the lysed samples at 37° for several hours to allow self-digestion by cellular proteases or digested the labeled peptides with Streptomyces griseus protease (data not shown). The use of relatively short pulse time and the sensitive analytical method in our approach, therefore, made it possible for us to examine in detail the newly synthesized proteins in response to amino acid deprivation. Our unpublished results also show that any change in the

length of pulse-labelling time from 30 sec. to 4 min. gave virtually identical gel patterns. The interpretation of our results is therefore not complicated by the breakdown of labeled peptides. We also avoided the use of temperature-shift experiment employed by Furano and Wittel (19) to achieve amino acid deprivation since a sudden change in temperature may have other effect on translation.

The gel analysis in Figure 2 also shows that several peptides, such as the 90 K, 59 K, and 50 K peptides, were induced in the rel$^+$ strain during leucine depriv tion. No peptides were induced in the rel A strain except that the synthesis of several peptides, such as the 130 K and 100 K peptides persisted for a long time aft removal of leucine (Fig. 2). It is unexpected that certain peptides are induced in response to amino acid deprivation since the translation apparatus of starved cells is presumably under stress due to the lack of an essential amino acid. The fact tha these prteins are indeed induced in the rel$^+$ strain as a result of leucine deprivati is supported by the observation that rifampicin, when added at the time of removal, blocked the appearance of these proteins and rendered the pulse-labeled peptide patt of the two strains identical (data not shown).

The function of the peptides that were induced during amino acid deprivation is no known. The results presented here raise the possibility that certain genes are turned on only in stringent strains of E. coli during amino acid deprivation and tha the products of these genes may well be related to the stringent control of protein breakdown in stringent strains of bacteria. The stringent control of protein breakdown during amino acid deprivation is in fact blocked by inhibitors of protein synth sis (5,8). Goldberg, however, disputed this notion and suggested that chloramphenic blocks protein breakdown not by inhibiting protein synthesis per se but by accumulat charged tRNA in the cell (6). More recently, Rafaeli-Eshkol and Hershko (8) showed that protein synthesis inhibitors which do not increase the level of charged tRNA su as puromycin, also block protein breaddown in starved cells. They actually suspecte that the formation of a rapidly-turning-over polypeptide may be necessary for the en hancement of protein breakdown in starved cells. Our observation on the induction o proteins in rel$^+$ cells in response to amino acid deprivation is in agreement with th

hypothesis. The possibility that the induced proteins may be related to cellular protein breakdown, or that one of the induced proteins may be a protease, in under investigation.

ACKNOWLEDGEMENTS

I thank B. Baggett and J.D. Karam for critical readings of the manuscript and helpful suggestions. The bacterial strains were kindly provided by P.P. Dennis.

This work was supported by NIH General Research Support Grant RR05420 to the Medical University of South Carolina, and by South Carolina State Appropriation for Biomedical Research.

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