ACTIVITY OF POLYPEPTIDE CHAIN INITIATION FACTORS DURING A NUTRITIONAL SHIFT-UP IN ESCHERICHIA COLI

STEPHEN M. BOYLE AND BRUCE H. SELLS

LABORATORIES OF MOLECULAR BIOLOGY

FACULTY OF MEDICINE

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

ST. JOHN'S, NEWFOUNDLAND, CANADA

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SUMMARY

During the first 10-15 minutes following nutritional shift-up in Escherichia coli, no significant increase was detected in the specific activity of initiation factors. The next 25 minutes, however, were characterized by a doubling of specific activity of these factors.

Measurements of initiation factor activity of different exponential growth rates revealed a linear proportionality between growth rate and initiation factor activity. For each doubling of growth rate, initiation factor activity increased 1.6-1.8 fold.

INTRODUCTION

During the first 15 minutes of nutritional shift-up - when bacterial cells are transferred from a poor to an enriched medium - there is an immediate increase in the rate of ribosomal protein synthesis and essentially no change in the rate of total protein synthesis (1, 2, 3, 4, 5). Previous studies from this laboratory which examined various components of the protein synthesizing system revealed that a 10 to 15 minute lag occurred before a measurable change was observed in the activity (6) and rate of synthesis of elongation factors (7). Because of the close parallel between the rate of total protein synthesis and elongation factor synthesis, the

results implied that elongation factor formation rather than ribosome formation regulates the overall rate of protein synthesis.

The present investigation of initiation factors was designed to determine:

1) whether changes in their specific activity paralleled those observed for elongation factors during nutritional shift-up, and
2) how their activity varied as a function of exponential growth rate.

MATERIALS AND METHODS

Bacterium and Culture Conditions

Escherichia coli H128 (leu^-, arg^-, thr^-, his^-, met^-, thi^-, rel^+) is a K-12 derivative (8) and was used in all these experiments. The cells were grown with shaking at 30°C in Cohen's salts basal medium (9) supplemented with a final concentration of 50ug/ml of the required amino acids, 2ug/ml of thiamine and 0.4% carbon source. Nutritional shift-up was effected in cells growing exponentially (OD_{575nm}=0.4) on potassium acetate by adding to the indicated final concentrations the following supplements: 0.4% glucose, 50ug/ml of the remaining 15 L-amino acids and 20ug/ml of the 5 nucleosides. Various growth rates, determined from the absorbance readings at 575nm, were obtained by growing the cells on their required supplements and either 0.4% proline, 0.4% potassium acetate, 0.4% glucose or 50ug/ml of 15 L-amino acids, 20ug/ml of 5 nucleosides and 0.4% glucose. The growth rates (mass doublings/hour) obtained were as follows: proline=0.20, acetate=0.44, glucose=0.81, glucose and amino acids and nucleosides=1.18.

Isotopes

(Methyl-^{3}H) l-methionine, specific activity 4.3 C/mM and H^3-L-leucine, specific activity 5.0 C/mM were obtained from New England Nuclear, Boston, Massachusetts.

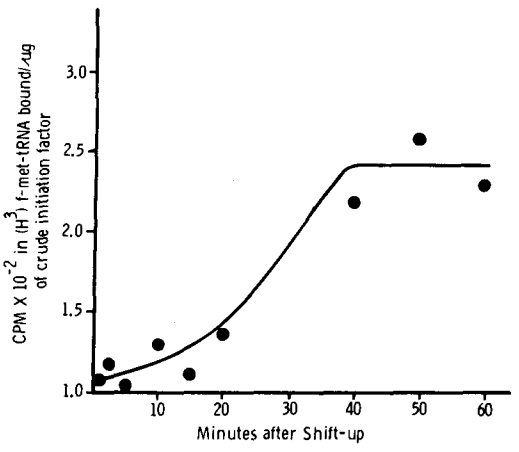

Figure 1 Specific activity of initiation factors assayed using f-met-tRNA binding system. At intervals during shift-up, 200 ml was removed from a 2 liter suspension of cells. The cells were poured over crushed ice, pelleted, washed with buffer, frozen in liquid nitrogen and stored at -70°C. Crude initiation factors were prepared within 1 to 2 days as described in Methods. Reactions were run at 25°C for 15 min with rate limiting amounts of crude initiation factors.

Preparation of f-(H^3)-met-tRNA

Formyl methionyl tRNA was prepared as described by Miller and Wahba (10) except that the source of $tRNA_f^{met}$ was Mann-Boeringher Company and the synthesized acylated tRNA was desalted on a G-25 column before being stored at -20°C. The specific activity of the formyl (H^3) methionyl-tRNA was 4620 counts x min^{-1} x $pmol^{-1}$ with 42% counting efficiency.

Cell Breakage, Initiation Factor Preparation

Cells, collected by pouring over an equal volume of crushed ice and pelleted by centrifugation, were suspended in Tris-HCl, pH7.8, 30mM NH_4Cl, 20mM Magnesium acetate, 1mM dithiothreitol and broken in

a French pressure cell at 10,000 p.s.i. The crude extract was then processed as described by Miller and Wahba (10) to obtain the 45-85% $(NH_4)_2SO_4$ fraction of the 1.0M NH_4Cl wash of the ribosomes, which contains IF1, IF2 and IF3 species, and was designated crude initiation factors.

Initiation Factor Assay

Initiation factors were assayed using the following two procedures:

a) Formyl-met-tRNA binding activity was performed as outlined by Dubnoff and Maitra (11). For each sample of crude initiation factor assayed, three different concentrations were employed to ensure that the factors were rate limiting.

b) In vitro protein synthesis was performed as outlined by Lee-Huang and Ochoa (12). The source of m-RNA was T_4 phage m-RNA extracted 8 min after infection of E. coli B, as outlined by Salser et al (13). For each sample of crude initiation factor assayed, 3 different concentrations were employed to ensure that the initiation factors were rate limiting. The cell-free protein synthesizing system was assayed for hot trichloroacetic acid precipitable C^{14}-labelled protein after 20 min at 37°C.

RESULTS

Specific Activity of Initiation Factors During Nutritional Shift-up

To determine the changes in the activity of the initiation factors during nutritional shift-up, two separate procedures were employed, each of which is dependent on the presence of all three (3) initiation factors. In Figure 1, the specific activity as measured by f-met-tRNA binding to ribosomes in the presence of the G-U-G codon is shown during nutritional shift-up. A delay of 15 to 20 minutes occurs before a significant increase in initiation factor activity is observed. After 40 to 45 minutes, the specific activity has reached a maximum characteristic of steady state growth in the shift-up medium (see data in Fig. 3). Similar data are

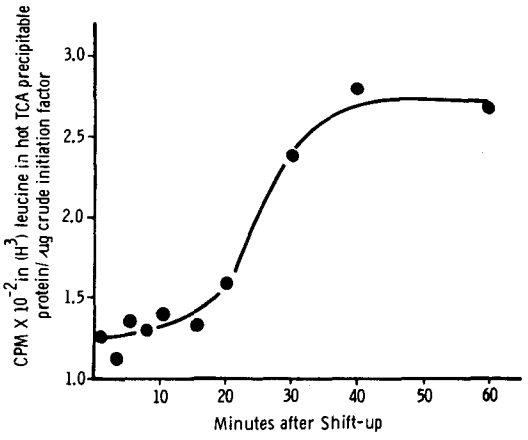

Figure 2 Specific activity of initiation factors assayed using T_4 Ø mRNA programmed in vitro protein synthesizing system. At intervals during shift-up 200 ml was removed from a 2 liter suspension of cells and poured over crushed ice. The cells were pelleted, washed with buffer, frozen in liquid nitrogen and then stored at -70°C. Crude initiation factors were prepared within 1 to 2 days after collection as described in Methods. Reactions were run at 37°C for 20 min with rate limiting amounts of crude initiation factors.

presented in Figure 2 where specific activity is measured by phage mRNA directed incorporation of radioactive-labelled amino acid into hot trichloroacetic acid precipitable material. These results confirm those presented in Figure 1. A 15 to 20 minute lag is observed before the specific activity changes, followed by an increase which reaches a maximum by 40 to 45 minutes.

Specific Activity of Initiation Factors at Various Growth Rates

Experiments were performed to examine the relationship between the specific activity of initiation factors and the growth rate of the bacterium. The rate of exponential growth was controlled by varying the carbon source

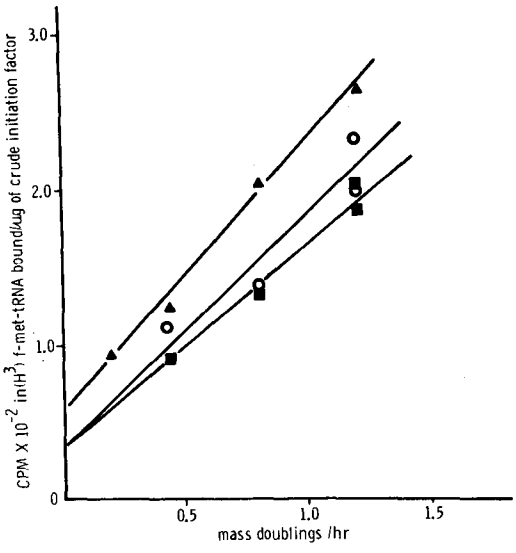

Figure 3 Relationship of initiation factor activity to mass doublings/hour. 200 ml cultures were grown at 30°C on various nutrient sources as described in Methods to an OD_{575nm}=0.5. The cells were then poured over crushed ice, pelleted, washed with buffer, frozen in liquid nitrogen and stored at -70°C. Crude initiation factors were prepared within 1 to 2 days after collection as described in Methods. Formyl-met-tRNA binding reactions were run at 25°C for 15 minutes with rate limiting amounts of crude initiation factors. -▲- Exp 1, -⊙- Exp. 2, -X- Exp. 3; Growth rates of 1.2 were obtained in Exp. 2 and Exp. 3 by growing cells on shift-up medium or nutrient broth; thus, there are two points for each curve at that growth rate.

present in the incubation medium as indicated in the Methods section. The results presented in Figure 3 are from three separate experiments. A linear relationship exists between specific activity and growth rate. A 2-fold increase in specific activity (i.e. 1.0 to 2.0) corresponds to a 2.5-fold to 3-fold increase in growth rate (doublings/hour).

DISCUSSION

These studies demonstrate that during nutritional shift-up, the specific activity of the initiation factors closely parallels the changes in the specific activity and rate of synthesis previously observed for elongation factors (6,7). This increase in initiation factor activity may not accurately reflect an increase in their rate of synthesis since interference factors specific for IF3 are present in the NH_4Cl washes of ribosomes used to prepare initiation factors (14, 15). In preliminary experiments (unpublished observations) designed to examine the rate of synthesis of the individual initiation factors during shift-up, we have examined the rate of synthesis of the proteins from the NH_4Cl wash of ribosomes. Five proteins migrating on polyacrylamide gels with the approximate Rf values of known initiation factors exhibit a 6 to 10-fold increase in their rate of synthesis by 20 minutes following shift-up.

The similarity between the sequence of changes in the specific activity of the initiation and elongation factors during nutritional shift-up suggests that both sets of proteins are regulated by a common mechanism. While elongation factors are present in one copy per ribosome (16), no such precise stoichiometry has been established for the various initiation factors. One study (17) estimates that in exponentially growing E. coli there are enough IF3 molecules for every 20 ribosomes or one IF3 for every two native 30S subunits. This fact argues against a common operon containing the cistrons for both initiation and elongation factors, unless there are multiple copies of elongation factor cistrons relative to the number of copies of initiation factor cistrons. This possibility will remain unresolved, however, until appropriate mutants for initiation factors are found and genetically and biochemically characterized. Studies are presently underway to measure the rate of synthesis of each of the initiation factors during nutritional shift-up to determine whether their

pattern of synthesis corresponds to the pattern observed for changes in their specific activity.

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