Regulation of Initiation and Elongation Factor Levels in Escherichia coli as Assessed by a Quantitative Immunoassay

(Received for publication, October 2, 1974)

SHARON WALD KRAUSS* AND PHILIP LEDER†
From the Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014

SUMMARY

These studies are directed toward determining whether the structural genes for protein biosynthetic factors comprise an operon subject to coordinate regulation in Escherichia coli. To assess coordinate expression of these genes, an immunoassay was devised to enable accurate quantitation of initiation and elongation factors in crude bacterial extracts. The antibodies made against highly purified initiation factor 2 (IF-2A and IF-2B) and elongation factor G (EF-G) are shown to inhibit the appropriate in vitro reactions and precipitate proteins co-migrating with appropriate factors on polyacrylamide gels. Immunoprecipitation in combination with gel electrophoresis was employed to make quantitative measurements of the amounts of IF-2 (A and B) relative to EF-G present in cells at different growth rates. The results show that the ratio of EF-G to IF-2 varies in a consistent way with the generation time of the cell. IF-2 levels remain constant as cells double more rapidly, whereas the EF-G content increases with more rapid cell growth.

The accurate translation of a messenger RNA requires the integrated activity of a variety of initiation, elongation, and termination factors (1). We wished to determine whether the genes for these factors might comprise an operon in Escherichia coli. This information would contribute to our understanding of the regulation of protein synthesis and may serve to focus the search and positive identification of the structural genes for these factors.

Experiments by Gordon (2) have indicated that regulation exists for the elongation factors to the extent that the amounts of EF-G and EF-Ts relative to ribosome numbers remain constant during different steady states of cellular growth. We have chosen to examine the relationship between the elongation factor EF-G and the initiation factor IF-2. To assess coordinate expression, an immunoassay was devised to enable accurate quantitation of initiation and elongation factors. The immunoassay enables one to determine the amounts of the relevant proteins in crude extracts where quantitative assay of specific biological activity is not possible. For the immunoassay extensive purification of the protein factors was necessary. In the case of IF-2, two separate species (IF-2A and IF-2B) were found that appeared to have no gross differences in biological function (3-9). Specific antisera were raised to IF-2A and IF-2B. These antisera inhibit the binding of fMet-tRNA to AUG but do not interfere with the poly(U)-dependent synthesis of polyphenylalanine. This indicates a specific inhibition of the initiation reaction in protein synthesis. Measurements by immunoassay of gene expression of EF-G and IF-2 were made under various physiological conditions, such as different growth rates and under inhibition of macromolecular synthesis. The results of these experiments are reported here and in the following paper.

EXPERIMENTAL PROCEDURE

Materials—Sodium [35S]sulfate having a specific activity ranging from 740 to 840 mCi/mmol and [14C]-labeled algal protein hydrolysate were obtained from New England Nuclear. Radioactive aminoacyl-tRNAs, poly(U), and AUG were prepared as described previously (6, 7). Polyacrylamide was from Polyscience; sodium dodecyl sulfate was from Sigma. Whatman DE52 DEAE-cellulose and Whatman P1 phosphocellulose were used in the preparation of the initiation factors.

Strains—Escherichia coli M 5073 (facile, gal E’K’/F’ gal’) was from Dr. E. Siger (9). E. coli MRE-600, originally isolated by Hayes, was harvested in early log phase for the preparation of initiation and elongation factors (10).

Media—“Modified M9” contained per liter: 1.0 g of NH4Cl, 0.2 g of MgCl2, 3.0 g of KH2PO4, and 6.0 g of Na2HPO4. This was supplemented with vitamin B1 (10 μg/ml) and sodium [35S]sulfate as indicated. The various carbon sources were as follows: glucose 1.0%, or 0.05% glycerol 0.4%, sucrose 0.6%, and pyruvate 0.2%. When [14C]-extracts were prepared, the cells were grown in M9, supplemented with vitamin B1, 1% glucose, and [14C]-labeled algal protein hydrolysate.

Elongation and Initiation Factor Assays—Polyphenylalanine synthesis reaction mixtures contained in 0.05 ml: 0.05 mM Tris-acetate, pH 7.2; 0.01 mM magnesium acetate, 0.05 mM NH4Cl, 5 mM GTP, 20 μg of poly(U); 1.0 A260 of ribosomes, 13 μg of EF-T; 0.5 μg of EF-G; and 20 pmol of [14C]labeled tRNA plus unlabeled aminoacyl-tRNAs. Incubation at 23° was stopped after 5 min by the addition of 1 ml of 10% Cl3CCOOH. The samples were heated at 90° for 15 min, cooled, and washed through Millipore filters with cold Cl3CCOOH. Determinations were in duplicate and counted in toluene-2,5-diphenyloxazole (PPO)-1,4-bis[2-(5-phenyloxazoyl)]benzene (POPOP).

The fMet-tRNA binding reaction mixture contained in 0.05 ml: 0.05 mM Tris-acetate, pH 7.2; 0.005 mM magnesium acetate; 0.05 mM NH4Cl; 0.001 M dithiothreitol; 5 mM GTP; 0.125 A450 of AUG, 20
pol of [3H]Met-tRNA plus 19 unlabeled aminoacyl-tRNAs; 1.0 A400 of six times NH4Cl-washed ribosomes. IF-2 was assayed by its ability to complement 1.55 µg of IF-1 in the AUG-dependent binding of [3H]Met-tRNA. Incubation was at 23°C for 15 min. The reaction was terminated by the addition of 1 ml of ice-cold buffer and the samples were washed through Millipore filters with cold buffer and counted in toluene-2,5-diphenyloxazole-1,4-bis(5-phenyloxazolyl)benzene.

Preparation of Initiation and Elongation Factors—All procedures were carried out at 4°C. One hundred fifty grams of frozen early log phase E. coli MRE-600 were thawed rapidly in 400 ml of a buffer containing 0.05 M Tris-acetate, pH 7.2, 0.01 m magnesium acetate, 0.05 M NH4Cl, and 0.001 M dithiothreitol. The suspension was passed twice through a French press at 10,000 p.s.i. and subjected to DNase (2 µg/ml) for 15 min. The supernatant was centrifuged for 30 min at 10,000 rpm and the supernatant was placed over 5 ml cushions of a buffer containing 0.01 M Tris-HCl, pH 7.4, 0.01 M magnesium acetate, 0.001 M dithiothreitol, and 10% glycerol. After centrifugation in a 60 Ti rotor at 50,000 rpm for 4 hours, the resulting pellets were resuspended in a buffer containing 0.05 M Tris-HCl, pH 7.4, 0.10 M NH4Cl, 0.01 M magnesium acetate, 0.011 M dithiothreitol, and 10% glycerol. The dialyzed preparation, “crude initiation factor,” was dialyzed for 6 hours against 3-liter batches of a buffer containing 0.05 M Tris-HCl, pH 8.0, 0.05 M KCl, 0.001 M dithiothreitol, and 10% glycerol. The dialyzed preparation, “crude initiation factor,” was dialyzed for 6 hours against 3-liter batches of a buffer containing 0.05 M Tris-HCl, pH 7.5, 0.05 M NH4Cl, and 0.001 M dithiothreitol. Subsequent purification of each IF-2 peak consisted of chromatography on twin phosphocellulose columns in the above buffer, using a linear salt gradient from 0.02 M to 0.5 M NH4Cl. Each IF-2 peak, as assayed by [3H]Met-tRNA binding, was concentrated by rapid freezing over a buffer containing 0.05 M Tris-HCl, pH 7.5, 0.05 M ammonium chloride, and 0.001 M dithiothreitol. The concentrated IF-2A and IF-2B samples were made 50%, in glycerol and applied to a Sephadex G-200 column that was eluted with a buffer containing Tris-HCl, pH 7.4, 0.05 M ammonium chloride, and 0.001 M dithiothreitol. The elution factors were purified from the S100 fraction according to Leder et al. (11).

Preparation of Antiserum—New Zealand white rabbits weighing about 2 kg were immunized in the foot pads with antigen emulsified in an equal volume of complete Freund’s adjuvant. After 30 days, each animal was bled from the central ear vein. If more serum was required, the rabbit was boosted intradermally with an antigen injection. The blood was allowed to clot, and the serum was collected by centrifugation and fractionated twice with 50% ammonium sulfate to elute. The concentrated IF-2A and IF-2B samples were made 50%, in glycerol and applied to a Sephadex G-200 column that was eluted with a buffer containing 0.05 M Tris-HCl, pH 7.5, 0.3 M ammonium chloride, and 0.001 M dithiothreitol. The elution factors were purified from the S100 fraction according to Leder et al. (11). The elution factors were purified from the S100 fraction according to Leder et al. (11).

Preparation of Antiserum—New Zealand white rabbits weighing about 2 kg were immunized in the foot pads with antigen emulsified in an equal volume of complete Freund’s adjuvant. After 30 days, each animal was bled from the central ear vein. If more serum was required, the rabbit was boosted intradermally with an antigen emulsified in incomplete Freund’s adjuvant and bled 1 week after injection. The blood was allowed to clot, and the serum was collected by centrifugation and fractionated twice with 50% ammonium sulfate at 4°C. The final precipitate was dialyzed against 0.05 M Tris-HCl, pH 7.5.

Increasing amounts of the γ-globulins were titrated against constant amounts of carrier initiation or elongation factors by dissolving in 0.1 N KOH the washed immunoprecipitates that had formed after incubation at 4°C overnight and determining their protein concentrations spectrophotometrically by the method of Murphy and Kies (12).

Immunoelectrophoresis—Immunoelectrophoresis was performed using a barbitol buffer pH 8.2 system. The samples were subjected to electrophoresis for 2 hours at 100 mv; the plates were developed with antisera either at room temperature for 3 hours or at 4°C overnight.

Sodium Dodecyl Sulfate-2-Mercaptoethanol-Polyacrylamide Gel Electrophoresis—The samples were prepared and subjected to electrophoresis at 100 volts for 16 hours at 4°C in a slab containing a linear 7 to 26% gradient of polyacrylamide. The procedures virtually identical with Maizel (13). Generally, the gels were pre-equilibrated to 4°C for at least 6 hours before the samples were applied.

Gel Assay of Initiation and Elongation Factors—An overnight culture of M 5073 was inoculated at a 100-fold dilution into 50 ml of modified M9 containing B1, a carbon source, and 2.5 µCi of Na23HPO4. The culture was allowed to grow at 37°C with aeration for approximately six generations until early log phase. The cell pellet was collected by centrifugation, washed twice in cold modified M9, and suspended in 1 ml of a buffer containing 2 mM ammonium chloride, 0.001 M dithiothreitol, 0.05 M Tris-HCl, pH 7.4, and 0.01 M EDTA. The suspension was sonicated in an ice bath for three 20-s intervals at 55 watts (Branson). The sonicates were stirred overnight at 4°C to elute ribosomal proteins. Cell debris was removed by centrifuging at 15,000 rpm in a Sorvall centrifuge and then the ribosomes were pelleted by centrifugation in a 40 rotor using 2 ml cellulose nitrate tubes and adapters for 2 hours at 39,000 rpm. The supernatant was used for immunosassay of initiation and elongation factors. Further washings of the pellet did not yield significant immunoprecipitable material.

After electrophoresis, the slab gels were dried on Miracloth and exposed to x-ray film (Kodak RPR-54) for a minimum of 8 hours. The appropriate areas of the gel were excised, rehydrated with 50 µl of distilled water, incubated at room temperature for 1/2 hour in 0.2 ml of hydrogen peroxide-2% ammonium hydroxide, placed at 60°C for 4 hours after the addition of 1 ml of NCS, cooled, and counted in 10 ml of toluene-2,5-diphenyloxazole-1,4-bis(5-phenyloxazolyl)benzene.

RESULTS

Purification of IF-2—When the ribosomal wash from Escherichia coli MRE-600 was chromatographed on DEAE-cellulose, two peaks of fMet-tRNA binding activity were found in the IF-2 region. These were called IF-2A and IF-2B, in order of their elution from DEAE. Each of these peaks was purified separately by phosphocellulose and Sephadex G-200 chromatography (“see Experimental Procedure”). The resulting Sephadex fractions of IF-2A and IF-2B were equivalent in their ability to stimulate fMet binding in the presence of the AUG codon. Fig. 1 shows the polyacrylamide slab gel electrophoresis of these Sephadex

![Fig. 1. Polyacrylamide gel electrophoresis of purified IF-2A and IF-2B. Sephadex fractions containing 50 units of activity were concentrated by trichloroacetic acid precipitation (13) and applied to a 7 to 26% gradient gel. Electrophoresis was at 100 volts for 16 hours at 4°C. A unit of activity is defined as the amount of factor capable of stimulating the binding of 1 pmol of fMet-tRNA in the presence of AUG.](http://www.jbc.org/)

Downloaded from http://www.jbc.org/ by guest on March 23, 2020
The effect of sera y-globulins on the poly(U)-dependent synthesis of polyphenylalanine. The assay was performed as described under "Experimental Procedure." The y-globulin was added to the assay mixture containing the salts, ribosomes, and poly(U) at 0°. EF-G and EF-T were added followed by GTP and aminoacyl-tRNAs. The reaction proceeded at 23°. A---A, anti-EF-G; A-A, normal serum; O-O, anti-IF-2A; O-0, anti-IF-2B.

Characterization of Antibody against Initiation Factors—The y-globulin fractions of antisera made against purified initiation factors were added to both AUG-fMet binding and poly(U)-polyphenylalanine assay systems in order to assess them functionally. In Fig. 2, the effect of antibodies against both initiation factors IF-2A and IF-2B and elongation factor EF-G in the poly(U)-dependent synthesis of polyphenylalanine is shown. While anti-EF-G inhibits the reaction, there is no inhibition of the reaction in the presence of either anti-IF-2A or anti-IF-2B. The stimulation above zero y-globulin and subsequent partial inhibition by the anti-initiation sera probably are nonspecific protein effects, since control y-globulin had an identical effect. The level of residual activity in the presence of anti-EF-G y-globulins is the same as that seen in a minus EF-G control.

The anti-initiation factor antibodies, however, were capable of inhibiting the AUG-dependent fMet-tRNA binding reaction (Fig. 3). In addition, both antibodies were potent against either the highly purified IF-2A or IF-2B samples; in other words, the cross-inhibition indicates that IF-2A and IF-2B are cross-reacting materials. Thus, antibodies against the initiation factors IF-2A and IF-2B specifically interfere with the initiation reaction of fMet-tRNA binding in the presence of AUG codon, but have no effect on the synthesis of polyphenylalanine in the presence of poly(U), a reaction known not to require initiation factors. Likewise, anti EF G y globuline inhibited only polyphenylalanine synthesis, dependent on the elongation reaction (11), but acted identically with control y-globulins in the fMet-tRNA binding initiation reaction (data not shown).

Reversibility of Inhibition by Active and Inactive IF-2—The inhibition of fMet-tRNA binding caused by anti-IF-2A or IF-2B y-globulins could be overcome by the addition of more IF-2. Interestingly, however, this inhibition was not affected by the addition of equal amounts of the same IF-2 preparation that had previously been completely inactivated by gentle heating (Fig. 4). There are a number of possible explanations for this. For instance, active IF-2 may not be displaced by inactive IF-2 because the antibody itself is "conformation-sensitive" or has altered affinity for inactive IF-2. Alternatively, the inactivated
Effect of anti-IF-2 on fMet-tRNA binding to factor-dependent and factor-independent ribosomes

Initiation factors and ribosomes were prepared as described under "Experimental Procedure." Factor-dependent ribosomes were washed six times with 1 M NH₄Cl. Factor-independent ribosomes were unwashed. The assay mixture containing the salts, ribosomes, and γ-globulins (if indicated) was prepared at 0°C. Initiation factors were added followed by GTP, AUG, and aminoacyl-tRNAs. The reaction proceeded at 23°C.

IF-2 could be incapable of competing with active IF-2 for ribosomal binding (see below).

Protection against Antibody Inhibition—Anti-IF-2 γ-globulins were capable not only of inhibiting the activity of highly purified IF-2A and IF-2B fractions that served as the immunizing antigen but also IF-2 from all previous stages of purification. However, no inhibition was found when the fMet-tRNA binding reaction proceeded in the presence of unwashed (initiation factor-independent) ribosomes (Table I). In other experiments, separate preincubation of the ribosomes with IF-1 and IF-2 plus each of the other elements required for fMet-tRNA binding did not prevent inhibition. Thus, IF-2 as it exists on ribosomes that have not been stripped of proteins during salt washes appears to be protected from antibody inhibition; that is, the antigenic site that renders IF-2 biologically inactive in combination with antibody in the presence of highly purified ribosomes is not accessible to the antibody on unwashed ribosomes.

Immunoelectrophoresis—The specificity of the antibody reaction was also characterized with respect to its immunoprecipitate. Immuneelectrophoresis was carried out with Sephadex-purified IF-2A and IF-2B fractions used for immunization. Only one precipitin line was visible (Fig. 5). However, when a crude initiation factor preparation was subjected to electrophoresis, several precipitin lines appeared, all of which had the opposite electrophoretic mobility from the Sephadex IF-2 (that is, they migrated toward the positive pole). Severely overloading the plate with crude IFs both with respect to protein and activity units did not bring out any minor components having the same mobility as the highly purified IF-2s. The DEAE-purified IF-2 fractions also migrated toward the positive pole and preincubation with GTP or with DEAE-purified IF-1 and IF-3 had no effect on their mobility. Similarly, mixing crude initiation factor preparations with highly purified IF-2 resulted in separate bands each at their characteristic positions. It appears that during purification some modification of IF-2 itself or an associated component results in a change in the electrophoretic mobility of immunoreactive IF-2.

Gel Analysis of Immunoprecipitates—In order to analyze the actual bacterial proteins precipitated with our various antisera, the immunoprecipitates themselves were collected, washed, and run on sodium dodecyl sulfate polyacrylamide slab gels. Fig. 6 shows the autoradiogram of such a gel. The samples applied were the immunoprecipitates resulting from the incubation of 14C-labeled crude bacterial extract (chromatographed on the left) and unlabeled carrier EF-G or initiation factors with various antisera. The predominant bands in each case coincide with authentic purified markers. To the right of the autoradiograms, a calibration curve of marker proteins indicates that the calculated molecular weight for IF-2A is 100,000 and for IF-2B 82,000. Bands corresponding to both species of IF-2 are present in the immunoprecipitate formed in the presence of either anti-IF-2A or anti-IF-2B, as would be expected from the previous cross-inhibition data (Fig. 3). In addition, the autoradiogram shows that immunoprecipitated EF-G is a distinct protein from the IF-2B band (see the mixture of anti-IF-2A and anti-EF-G precipitates). Thus, with the immune sera, one can specifically isolate a single protein from out of the large spectrum of protein bands visible in a crude bacterial extract. Other bands do appear on the gels when the immunoprecipitates are subjected to electrophoresis. Some of these are due to nonspecific denaturation of proteins in the labeled extract (see normal serum and no serum) or to persistent adsorption on sera proteins despite washes in the presence of Triton X-100. Some auxiliary bands are characteristic of a particular

| Addition                                      | pHJfMet-tRNA bound |
|----------------------------------------------|--------------------|
| Factor-independent ribosomes                 | 1.0                |
| Factor-independent ribosomes plus control γ-globulin | 1.31              |
| Factor-independent ribosomes plus anti-IF-2 γ-globulin | 1.20              |
| Factor-dependent ribosomes plus IF-1 and IF-2 | 2.02              |
| Factor-dependent ribosomes plus IF-1 and IF-2 and control γ-globulin | 2.18              |
| Factor-dependent ribosomes plus IF-1 and IF-2 and anti-IF-2 γ-globulin | 0.45              |
antiserum. For instance, in the immunoprecipitates of anti-IF-2A, there is always a large band in the 50,000 molecular weight region. This could represent a cross-reacting structure of physiological significance or merely a contaminating antigen in our original purified IF-2A (Fig. 1). Because after immunoprecipitation a major radioactive protein band can be recovered at the positions identical with purified EF-G, IF-2A, or IF-2B factors, it was possible to devise a quantitative assay for these factors. For such an assay, one must be in the region of slight antibody excess to ensure complete precipitation of the experimental sample. Accordingly, each antibody preparation was calibrated against either unlabeled crude initiation factors or S100. Levels of carrier proteins for the immunoprecipitation assay were carefully chosen so that while a visible precipitate was formed, there was adequate antibody present to precipitate completely the relevant protein in the labeled extract. In addition, assays were always performed at two concentrations of labeled extract to be certain of not being in the region of antigen excess.

A standard curve for the quantitative assay of EF-G factor by the immunoprecipitate-gel electrophoresis technique is shown in Fig. 7. For this experiment various amounts of radioactive extract were incubated with a constant amount of unlabeled S100 carrier and anti-EF-G y-globulin. The amount of counts per min recovered from the EF-G band on the gel is linear with respect to the volume of extract assayed. The autoradiogram of the supernatant from such an immunoprecipitation reaction mixture contained a large number of proteins but the EF-G band was eliminated. Also, controls showed that none of the labeled proteins which precipitate in the presence of nonimmune y-globulins corresponded to EF-G. Thus, using techniques that combine specific immunoprecipitation with subsequent gel electrophoresis of the reaction products, one can accurately quantitate the amounts of EF-G, IF-2A, and IF-2B that are present in a given crude bacterial extract.

Expression of Factors at Different Growth Rates—With quantitative assays for a representative elongation factor and representative initiation factors at hand, it was possible to measure the relative levels of their expression in various circumstances. It is well known that the ribosomal content of bacterial cells varies with generation time, and previous work by Gordon (2) proposed that the amounts of the elongation factors EF-G and EF-T relative to ribosomes remained constant at different steady states of growth. Therefore, it seemed reasonable to compare the amounts of initiation factors IF-2A and IF-2B in cells with different generation times to see if they followed the pattern of the elongation factors. Bacterial strain M 5073 was grown on various carbon sources in the presence of 14C and assays for the factors were performed on crude extracts from these cells (see “Experimental Procedure”). The results of a series of such experiments are shown in Fig. 8. When the ratio of EF-G to IF-2 (A and B) is plotted against generation time, a straight line is obtained; the ratio of these factors decreased with decreasing rate of growth. Plotted below are the same data expressed as picomoles per cell as a function of generation time. The amount of IF-2 (A and B) remained fairly constant while the amount of EF-G per cell varied over a 10-fold range depending on the generation time.

DISCUSSION

Properties of Purified Initiation Factors—A number of the factors involved in the protein synthetic process have now been extensively purified. EF-G has been isolated in crystalline form (14). IF-2, on the other hand, is rather difficult to work with; it adheres to glass and is extremely sensitive to inactivation. Indeed, we found that it was essential to perform all of the...
necessary purification procedures as rapidly as possible. In our laboratory, as well as several others (3-5), it was recognized that the peak of IF-2 activity obtained during fractionation of crude initiation factors contained two distinct species of biologically active molecules (IF-2A and IF-2B). In our hands, the two peaks had about equal activity in the stimulation of fMet-tRNA binding and there was sometimes a small additional peak which had about equal activity in the stimulation of fMet-tRNA binding.

FIG. 8. Expression of EF-G and IF-2 at different rates of growth. The immunoprecipitate-gel electrophoresis assay was performed on 35S-labeled crude bacterial extracts from cells grown on glucose, glycerol, succinate, or pyruvate as their sole carbon source. Top, the ratio of EF-G:IF-2 (A and D) in picomoles per cell is plotted as a function of generation time. Bottom, the same data is presented, expressed as picomoles of a given factor per cell as a function of generation time. All assays were performed in duplicate and at two concentrations of %-labeled crude bacterial extracts. O--O, EF-G; 0--0, IF-2 (A and B).

Thus far the significance of the two forms is unknown, but it is likely that IF-2A and IF-2B have a precursor-product relationship. Many careful studies have been made evaluating differences in function which might exist and the results are conflicting. Mazumder (3) reported that one of his two peaks of IF-2 activity did not promote fMet-tRNA complex formation with AUG, GUG, or MS2 RNA at 37°C. Miller and Wahba (5) found that both their proteins promoted initiation complex formation with AUG, GUG, or R17 RNA and fMet-tRNA at 25°C but that both had relatively little activity at 37°C.

Another unresolved question is that of the mode of interaction of IF-2 with other components of the initiating system, such as ribosomes and GTP. We have shown that crudely isolated ribosome-bound IF-2 is not subject to inhibition by IF-2-specific antibodies. The immunoglobulins prevent IF-2-dependent fMet-tRNA binding only after purified IF-2 is added back to ribosomes that have been extensively salt-washed. The basis for the protection of IF-2 from antibody inhibition on unwashed ribosomes remains to be defined. Our immunoelectrophoretic data indicate that crude IF-2 seems to be associated with something that confers a negative charge. Reports on the possible interaction of IF-2 with GTP have been controversial. It had been observed that IF-2 was protected by GTP from inhibition by sulphydryl-binding reagents (3) and against heat inactivation (15). A stable complex of fMet-tRNA, GTP, and IF-2 had also been reported (16, 17). However, Miller and Wahba (5) found neither IF-2A nor IF-2B to be protected by GTP against heat inactivation, and neither protein bound [y-32P]GTP in the presence of fMet-tRNA. We found that a simple incubation of purified IF-2 with GTP did not regenerate the species having the original immunoelectrophoretic mobility nor could we find any species of immunoreactive material labeled in vivo in a crude bacterial extract that was grown in the presence of [32P]A.

Characteristics of Specific Immunooassay—Because the mechanism of IF-2 action and its physiology are not understood, the immunoassay technique described here, employing analysis of the precipitate by gel electrophoresis, offers advantages when compared with other immunological procedures. Leder et al. (8) used a quantitative radial immunodiffusion technique to determine that EF-G comprises more than 2% of the soluble protein of log phase E. coli. A complement fixation assay was employed by Scheps and Revel (18) to compare the amount of IF-3 in growing and nongrowing cells. Gordon (2) used a turbidimetric immunoassay to follow the amounts of EF-G during different growth rates. These methods have a major feature in common: biological activity of the factor is not required. Any partial forms of the proteins, be they precursors or breakdown products, of course, could give an immunological reaction with the antibody. These cross-reacting materials might significantly contribute to other immunoassays that depend on the total amount of material that can be bound by the immunoglobulins. However, in our studies we are specifically looking at only the mature protein factor, a form that is known to have biological activity. In addition, although we have demonstrated the specific inhibition of particular biological reactions with our antisera, the fact that the immunizing antigens contained small amounts of non-IF polypeptides (Fig. 1) does not invalidate our method, because we are concerned only with the assay of protein bands that coincide with the authentic marker proteins EF-G, IF-2A, and IF-2B.

Specific antibodies are not only valuable to quantitate the factors in crude extracts but also as a reagent with which to study their physiologic actions. Antibodies can be used to probe the mechanisms of action of IF-2 in relation to IF-1 and IF-3 in the binding of fMet-tRNA to ribosomes. In addition, specific antibodies against the factors can be used to clarify controversial aspects of the ribosomal cycle. Also, the relationship of the factors to the cell cycle is amenable to investigations using immunological techniques. A decrease in IF-3 as the cell enters stationary phase already has been shown (18). Finally, antibodies are a great potential interest to the study of the effects of phage infection on the protein synthetic apparatus of the host. In the case of phage T4 (19) and phage T7 (20, 21), alterations of initiation factors resulting from phage infections have been reported. In preliminary experiments, we have found a decrease in total IF-2 after infection with phage T7 in an F÷ strain.

3 S. W. Krauss and P. Leder, unpublished observations.
4 S. W. Krauss and P. Leder, unpublished results.
Regulation of Initiation and Elongation Factors in Growing Cells—Our measurements of the steady state levels of EF-G and IF-2 (A and B) during different rates of growth provide evidence that there is regulation in the cell of these factors. We found that there is a unique ratio of the EF-G to IF-2 factors, which varies in a consistent way with the generation time of the cell such that as the doubling time decreases more EF-G is present in the cell while the IF-2 level remains relatively constant. This regulation, however, is not of the classical operon type where translation occurs on a single messenger RNA. In that situation, while there may be unequal molar amounts of the structural gene products, their ratios would not vary even though the total amounts may be different under different conditions. Therefore, we would predict on the basis of our results that the initiation factors and elongation factors would not necessarily map together. Indeed, recent evidence (22) suggests that the structural genes for EF-G and EF-Ts have different chromosomal locations even though Gordon’s previous data (2) indicate that synthesis of both the EF-G and EF-T factors are coordinated with ribosomes. Perhaps one key to the organization of the structural genes for initiation, elongation, and termination factors will lie in more complete understanding of all of the reactions in which those factors participate. For example, Blumenthal et al. (23) found that the replicase of bacteriophage Qβ contains the EF-Tu and EF-Ts factors. Thus, for these genes, at least, there may exist a particular organization, which will allow their regulated expression in one situation (e.g. adjustment to various growth rates), while enabling independent expression of only certain of these genes at other times (e.g. Qβ infection).

Acknowledgments—We are grateful to Dr. Michael Mage for guidance in immunoelectrophoresis and to Dr. Howard Nash for advice about sonication. Our thanks also to Barbara Norman for technical suggestions and to Catherine Kunkle for assistance in the preparation of this manuscript.

REFERENCES

1. Haselkorn, R., and Rothman-Denes, L. B. (1973) Ann. Rev. Biochem. 42, 397-438
2. Gordon, J. (1970) Biochemistry 9, 912-917
3. Mazumder, R. (1971) FEBS (Fed. Eur. Biochem. Soc.) Lett. 16, 64-66
4. Fakunding, J. L., and Hershey, J. W. B. (1973) J. Biol. Chem. 248, 4206-4212
5. Miller, M. J., and Wahba, A. J. (1973) J. Biol. Chem. 248, 1084-1090
6. Roufa, D. J., and Leder, P. (1971) J. Biol. Chem. 246, 3100-3107
7. Erbe, R. W., Nau, M. M., and Leder, P. (1969) J. Mol. Biol. 38, 441-460
8. Leder, P., Skogerson, L. E., and Roufa, D. J. (1969) Proc. Natl. Acad. Sci. U. S. A. 62, 928-933
9. Dinsen, E. (1967) Virology 33, 352-354
10. Cammack, K. A., and Wade, H. E. (1965) Biochem. J. 96, 671-680
11. Leder, P., Skogerson, L. E., and Nau, M. M. (1969) Proc. Natl. Acad. Sci. U. S. A. 62, 454-460
12. Murphy, J. B., and Kies, M. W. (1960) Biochim. Biophys. Acta 45, 382-384
13. Mazel, J. V. (1971) in Methods in Virology (Maramorosch, K. and Koprowski, H., eds) Vol. 5, pp. 180-246, Academic Press, New York
14. Kaziro, Y., Inoue-Yokosawa, N., and Kawakita, M. (1972) J. Biochem. 72, 853-863
15. LeLong, J. C., Grunberg-Manago, M., Donlon, J., Gros, D., and Gros, F. (1970) Nature 226, 505-510
16. Redland, P. S., Weydrow, W. A., and Clark, B. F. C. (1971) Nat. New Biol. 231, 76-78
17. Lockwood, A. H., Chakraborty, P. R., and Maitra, U. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 3122-3126
18. Scheff, R., and Revel, M. (1972) Eur. J. Biochem. 29, 319-325
19. Hsu, W., and Weiss, S. B. (1969) Proc. Natl. Acad. Sci. U. S. A. 64, 345-351
20. Leder, P., Skogerson, L. E., and Callahan, R. (1972) Arch. Biochem. Biophys. 153, 814-822
21. Scheff, R., Zellee, H., and Revel, M. (1972) FEBS (Fed. Eur. Biochem. Soc.) Lett. 17, 1-4
22. Gordon, J., Baron, L., and Sweiger, M. (1972) J. Bacterial. 110, 306-312
23. Blumenthal, T., Landers, T. A., and Weber, K. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 1919-1927
Regulation of initiation and elongation factor levels in Escherichia coli as assessed by a quantitative immunoassay.
S W Krauss and P Leder

J. Biol. Chem. 1975, 250:3752-3758.