Function of Initiation Factor 1 in the Binding and Release of Initiation Factor 2 from Ribosomal Initiation Complexes in Escherichia coli

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1. Studies on the function of initiation factor 1 (IF-1) in the formation of 30 S initiation complexes have been carried out. IF-1 appears to prevent the dissociation of initiation factor 2 (IF-2) from the 30 S initiation complex. The factor has no effect on either the initial binding of IF-2 nor does it increase the amount of IF-2-dependent fMet-tRNA and GTP bound to the 30 S subunit. Bound fMet-tRNA remains stable to sucrose gradient centrifugation even in the absence of IF-1.

2. It is postulated that the presence of IF-2 on the 30 S complex is necessary so that at the time of junction with the 50 S subunit to form a 70 S complex, the 70 S-dependent GTPase activity of IF-2 can hydrolyze GTP. This hydrolysis provides a means by which GTP can be removed to facilitate formation of a 70 S initiation complex active in peptidyl transfer. In support of this postulate, it was observed that 30 S initiation complexes formed in the absence of IF-1 could be depleted of their complement of GTP and IF-2. Such depleted initiation complexes were still able to accept 50 S subunits to form 70 S complexes which could still donate fMet-tRNA into peptide linkages. These results indicate that 30 S complexes lacking GTP do not require IF-2 for formation of active 70 S complexes.

3. IF-1, which is required to prevent dissociation of IF-2 from the 30 S initiation complex, is also required for release of IF-2 from ribosomes following 70 S initiation complex formation. The mechanism of the release of IF-2 has been studied in greater detail. Evidence is presented which rules out the presence of a stable IF-2-GDP complex on the surface of the 70 S ribosome following GTP hydrolysis and of any exchange reactions between IF-1 and guanine nucleotides necessary for effecting the release of IF-2. IF-2 remains on the 70 S initiation complexes after release of guanine nucleotides and can be liberated solely by addition of IF-1.

Initiation of protein synthesis in Escherichia coli requires three protein factors, initiation factors 1, 2, and 3, and leads to the GTP-dependent binding of fMet-tRNA to ribosomes to form the 70 S initiation complex (1). The roles of two of these factors, IF-2 and IF-3, have been well documented. IF-2 is necessary for the binding of both fMet-tRNA and GTP to the 30 S ribosomal subunit to form the 30 S initiation complex. IF-2 has a ribosome-dependent GTPase activity which catalyzes the hydrolysis of GTP following junction of the 50 S subunit to the 30 S initiation complex to form the 70 S initiation complex (2-4). IF-3 is necessary for the recognition of natural messenger RNA (5) and has an antiribosomal subunit association activity which ensures a constant pool of 30 S subunits (6-8). The function of IF-1 in the initiation process is less clear. IF-1 is not necessary for formation of a functional 70 S initiation complex since fMet-tRNA is bound to the ribosomal P-site of the ribosome even in its absence (9). However, IF-1 stimulates 70 S initiation complex formation severalfold. Recent studies from this and other laboratories (9, 10) have demonstrated that IF-1 is necessary for the release of IF-2 from the 70 S initiation complex thus allowing recycling of IF-2 and conversion of the reactions leading to initiation complex formation from stoichiometric to catalytic reactions. These results explain the stimulatory effect of IF-1 in 70 S initiation complex formation. At the level of 30 S initiation complex formation, the function of IF-3 remains ill defined. Chao et al. (11) reported that at 25°C, IF-1 stimulated IF-2-dependent binding of fMet-tRNA to 30 S ribosomes severalfold, presumably by increasing the stability of ribosome-bound fMet-tRNA, while at 0°C, the factor had no effect. Benen et al. (12), however, observed no significant stimulation of fMet-tRNA binding to the 30 S initiation complex by IF-1. In other studies, IF-1 has also been implicated in stabilizing and increasing the affinity of messenger RNA binding to ribosomes (13, 14). It has also been reported (15) that IF-1 enhances the effects of IF-3 in maintaining a pool of 30 S subunits and in stabilizing the binding of IF-2 to the 30 S subunit (16, 17).

In the present communication, we have carried out a further detailed investigation of the role of IF-1 in formation of initiation complexes. We show that while IF-1 does not stimulate the binding of fMet-tRNA and GTP to 30 S subunits, it plays an important role in stabilizing the binding of IF-2 to the 30 S initiation complex in the presence of other components of the reaction (IF-3 has no effect). The presence of IF-2 on the 30 S complex is necessary since its 70 S-dependent GTPase activity

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have found that IF-1 alone is required for release of IF-2 and junction of the 30 S complex with the 50 S subunit to form a 70 S reaction mixtures.

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buffer, pH 7.0, 2 mM 2-mercaptoethanol, 5 mM magnesium acetate, charged lSHlmethionine, 1300 cpm/pmol). Unless otherwise indicated, the membranes were washed with 6 ml of this buffer, dried, and filtered through nitrocellulose membranes under reduced pressure.

was present in the reaction mixture. The diluted solutions were used was similar to that described for coupled fMet-tRNA binding communications from this laboratory (10).

Materials-The preparation of labeled [Met-tRNA (labeled with [methyl-3H]methionine, 1300 cpm/pmol), or [methyl-14C]methionine, 90 cpm/pmol], and purified initiation factors 1, 2, and 3 were as described by Dubnoff and Maitra (19).

The specific activities of IF-1, IF-2, and IF-3 were on the average 40,000 units, 100,000 units, and 40,000 units, respectively, per mg of protein; 1 unit of activity is equal to 1 pmol of fMet-tRNA bound to ribosomes under the conditions of the assay previously described (19). Other characteristics of purified factors were as described previously (19). Salt-washed ribosomes, free of GTPase activity, were prepared by a modification of the method of Stachelin and Maglott (20) in which DNase-treated S-30 was layered over 1.1 M sucrose solution containing 20 mM Tris/HCl buffer, pH 7.8, 0.5 mM NaCl, 10 mM magnesium acetate, and 5 mM 2-mercaptoethanol (Buffer A) and was centrifuged for 16 h in a Spinco No. 42 rotor. The clear ribosomal pellet was suspended in Buffer A containing 0.5 mM magnesium acetate and were dissociated into subunits by dialysis against Buffer A containing 0.5 mM magnesium acetate. The ribosome pellets were suspended in 20 mM Tris/HCl buffer, pH 7.8, 10 mM magnesium acetate, 0.1 M NaCl, and 40% glycerol and stored at -20° at a concentration of 1,000 A$_{260}$ units/ml. The individual ribosomal subunits were prepared by the method of Stachelin and Maglott (20). The 30 S ribosome subunits were heat-activated before use (21).

The sources of all other reagents were as described in previous communications from this laboratory (10).

Assay of [Met-tRNA Binding to Ribosomes-The method of assay used was similar to that described for coupled fMet-tRNA binding and GTP hydrolysis (3), and it measured retention of labeled fMet-tRNA on nitrocellulose membrane filters in the presence of other initiation components. Reaction mixtures (0.125 ml) contained 50 mM Tris/HCl buffer, pH 7.0, 2 mM 2-mercaptoethanol, 5 mM magnesium acetate, 80 mM NaCl, 200 µM GTP, 2 A$_{260}$ units of poly(U), and [fMet-tRNA (containing 20 pmol) of charged [H]methylmethionine, 1,300 cpm/pmol]. Unless otherwise indicated, initiation factor additions were 0.15 µg each of IF-1 and IF-3 and 0.05 µg of IF-2. Incubation was for 15 min at 25° after which reaction mixtures were chilled and diluted with 3 ml of cold Tris buffer, Mg$^{++}$, and Na$^+$ buffer solution of the same composition as was present in the reaction mixture. The diluted solutions were filtered through nitrocellulose membranes under reduced pressure. The membranes were washed with 6 ml of this buffer, dried, and counted in a liquid scintillation counter.

RESULTS

Effect of IF-1 on Binding of IF-2 to 30 S Initiation Complex-IF-2 promotes the formation of the 30 S initiation complex. The 30 S initiation complex contains bound fMet-tRNA, GTP, mRNA, IF-2, and IF-1 (1). The following series of experiments were performed to examine the influence of IF-1 on the binding of IF-2, and of fMet-tRNA and GTP to the 30 S subunit.

In these experiments, initiation reaction mixtures were prepared with 30 S subunits. Following 30 S initiation complex formation, reaction mixtures were layered onto sucrose gradients and centrifuged. The position of IF-2 was determined by monitoring its activity in the binding of fMet-tRNA$_{\text{rev}}$ to ribosomes.

Fig. 1A shows that IF-2 activity could only be detected in the region of the 30 S subunit when the 30 S complex was formed in the presence of IF-1. In contrast, when 30 S complexes were formed in the absence of IF-1, IF-2 activity was detected at the top of the gradient (Fig. 1B). Since 30 S initiation complexes which are formed with synthetic messenger RNAs do not require IF-3 (4, 5), it was possible to show that the stability of IF-2 binding was due specifically to IF-1. In the experiment presented in Fig. 1C, 30 S initiation complexes were formed in the absence of IF-1.
using poly(U,G) as messenger and even though IF-3 was absent, IF-2 remained bound to the 30 S subunit when IF-1 was present. In all cases, no matter whether IF-2 remained bound to or was released from the 30 S complex in the presence or absence of IF-1, fMet-tRNA remained firmly bound to the 30 S subunit (Fig. 1).

When initiation complexes were formed in the absence of IF-1 with a mixture of 30 S and 50 S subunits, IF-2 remained bound to the 70 S initiation complex formed (Fig. 2A). However, when 30 S complexes were formed first and subsequently incubated with 50 S subunits (Fig. 2B), IF-2 was released and sedimented at the position of free factor. On the other hand, fMet-tRNA is found on the 70 S ribosome irrespective of the time of addition of 50 S subunits to the 30 S initiation complex (Fig. 2, A and B). These experiments suggest that in the absence of IF-1, 50 S subunits must be present initially so that a stable 70 S complex containing IF-2 can be formed. Under these conditions, IF-2 does not have an opportunity to be released from the 30 S complex as is the case when 50 S subunits are added after the 30 S complex has formed.

Thus, these results indicate that IF-1 stabilizes IF-2 binding in a manner which is independent of IF-3. In the absence of IF-1, IF-2 binding may also be stabilized as part of the 70 S complex provided 50 S subunits are present at the beginning of the initiation reactions.

The effect of IF-1 on the binding of fMet-tRNA and of GTP to the 30 S subunit was also measured (Fig. 3). The 30 S initiation complexes were formed with [3H]Met-tRNA and [γ-32P]GTP in the absence of IF-1 and the amounts of these labeled components bound to the complex was analyzed by Sephadex G-100 gel filtration. The same amount of GTP and fMet-tRNA remained bound to the isolated 30 S complex as that found in isolated complexes formed in the presence of IF-1. It is to be noted that the appearance of 3H and 32P radioactivity in the void volume of the Sephadex G-100 column was completely dependent upon the presence of 30 S subunits (data not shown). In the experiment presented in Fig. 3, the amount of fMet-tRNA and GTP bound to the 30 S subunits in the presence of IF-1 were calculated to be 6.6 and 7.8 pmol, respectively, while in the absence of IF-1, these values were 6 and 8 pmol, respectively. Thus, there was approximately a 1:1 ratio of bound GTP to bound fMet-tRNA showing that even though IF-1 stabilizes IF-2 binding it neither stimulated the amount of fMet-tRNA bound nor enhanced GTP binding.

Properties of IF-2-deficient 30 S Initiation Complex – Results presented in the previous section demonstrated that even under conditions where IF-1 is not present and IF-2 does not remain bound to 30 S complexes, fMet-tRNA remained bound to 50 S subunits. It was of interest to determine whether a 70 S initiation complex active in peptidyl transfer could be formed by addition of 50 S subunits to an IF-2-deficient 30 S complex. For this purpose it was necessary to prepare a 30 S complex deficient in IF-2 as well as GTP since the presence of GTP in the 70 S complex interferes with the subsequent puromycin reaction (9, 16, 17). To prepare a GTP-deficient 30 S initiation complex, we took advantage of our previous observation (4) that incubation of isolated 30 S initiation complexes (containing both bound fMet-tRNA and [γ-32P]GTP) in a ratio of 1:1 for 15 min at 30° resulted in the release of most of the bound GTP without substantially decreasing the level of bound fMet-tRNA. [γ-32P]GTP released from the 30 S complex in this manner had not been hydrolyzed, since the released 32P was still Norit-adsorbable and acid-soluble (4). As shown in
Fig. 4, when a 30 S initiation complex was prepared with \( f^3H \)Met-tRNA and \( \gamma^32P \)GTP in the absence of IF-1 and then incubated at 30°C for 15 min and centrifuged through a sucrose gradient. Most of the IF-2 and GTP was released and sedimented at the top of the gradient while the initiation complex contained bound Met-tRNA. Such an isolated 30 S complex, deficient in both IF-2 and GTP, was able to produce puromycin reactive fMet-tRNA after incubation with 50 S subunits (Table I). This shows that once the 30 S initiation complex has formed, neither IF-2 nor GTP need be present on the 30 S complex when junction with the 50 S subunit occurs for an active 70 S complex containing fMet-tRNA in the ribosomal P-site.

**Effect of IF-1 on Release of IF-2 from 70 S Initiation Complex**

![Diagram](https://via.placeholder.com/150)

**Fig. 4. Isolation of an IF-2- and GTP-deficient 30 S initiation complex.** The experiment was carried out in two stages. The first stage involved isolation of a 30 S initiation complex containing bound fMet-tRNA and \( \gamma^32P \)GTP, while the second stage involved depletion of bound GTP from the 30 S complex by sucrose gradient centrifugation. An initiation reaction mixture (0.2 ml) was prepared as described in legend to Fig. 1 except that 1.5 nmol of \( \gamma^32P \)GTP was used instead of GTP. An initiation reaction mixture (0.2 ml) was prepared as described in legend to Fig. 1 except that 1.5 nmol of \( \gamma^32P \)GTP (4000 cpm/pmol), 75 pmol of \( f^3H \)Met-tRNA, 2.7 A\textsubscript{260} units of 30 S ribosome, 55 units of IF-2, and 25 units of IF-3 were added. After incubation at 30°C for 15 min, the reaction mixture was chilled and applied to a column (20 x 0.5 cm) of agarose 1.5m which had been equilibrated with TMA buffer at 5°C. The peak of \( f^3H \) and \( \gamma^32P \) radioactivity which appeared in the excluded volume was collected and pooled to obtain the 30 S initiation complex which contained \( f^3H \)Met-tRNA and \( \gamma^32P \)GTP in approximately equimolar amounts. After isolation of the 30 S complex was incubated for 15 min at 30°C to release bound GTP (4). An aliquot of 0.2 ml was taken and layered onto a 5-20% (w/v) sucrose gradient and centrifuged as described in legend to Fig. 1 except that GTP was not included in the sucrose gradient. Fractions of 0.2 ml were collected and \( \beta^32P \) radioactivity due to \( f^3H \)Met-tRNA and \( \beta^3P \) due to \( \gamma^32P \)GTP were determined by counting aliquots in Bray’s solution. Gradient fractions were also assayed for IF-2 activity as described in the legend to Fig. 1. ○ ○ ○ ○, \( f^3H \)Met-tRNA; ○ ○ ○ ○, \( \gamma^32P \)GTP; ●●●●, IF-2 activity.

**Table I**

| Additions          | f^3H Met-tRNA retained in complex | f^3H Met-puromycin formed |
|--------------------|----------------------------------|--------------------------|
| 30 S complex       | 14.0                             | <0.1                     |
| 30 S complex + 50 S| 13.6                             | 9.0                      |

**Reactions of IF-2- and GTP-deficient 30 S initiation complex**

The peak fractions of 30 S region following sucrose gradient analysis (as described in legend to Fig. 4) were pooled. The isolated material was divided into 0.2 ml aliquots. Each aliquot contained 14 pmol of \( f^3H \)Met-tRNA, <1 pmol of \( \gamma^32P \)GTP, and <1.5 units of IF-2. Aliquots of 0.2 ml were used and were supplemented with 2.5 A\textsubscript{260} units of 50 S subunits and 50 nmol of puromycin as indicated. After incubation for 10 min at 30°C, fMet-tRNA binding was measured by Millipore filtration while \( f^3H \)Met-puromycin formation was measured according to the method of Leder and Bursztyn (22).

The results presented in Fig. 5 show that without any further addition to the isolated 70 S complex (in the incubation mixture), IF-2 remained bound to the ribosome (Fig. 5A). When IF-1 alone was added to the incubation mixture, >95% of the IF-2 activity was released from the 70 S ribosomal complex and sedimented at the position of free factor (Fig. 5B). Addition of GTP or GDP along with IF-1 had no stimulatory effect on IF-2 release (data not shown). These results show that the release of IF-2 from the 70 S initiation complex depended only on the presence of IF-1.

The question was also asked whether in the absence of IF-1, IF-2 remained on the 70 S initiation complex as an [IF-2·GDP] complex. Since IF-1-dependent release of IF-2 from the 70 S initiation complex requires GTP hydrolysis, it has been postulated (12) that IF-2 may be released as an [IF2·GDP] complex. Therefore in the absence of IF-1, the [IF2·GDP] complex should remain on the ribosome. Thus, if a 70 S initiation complex is formed under "coupled" conditions (3) where 1 pmol of fMet-tRNA\textsubscript{new} is bound to the P-site on the ribosome for each picomole of GTP hydrolyzed, the ratio of fMet-tRNA\textsubscript{new} to GDP bound to the ribosomes should equal parity in the absence of IF-1. We have attempted to determine this ratio by carrying out initiation reactions with \( f^3H \)Met-tRNA and \( \alpha^32P \)GTP in the absence of IF-1 followed by analysis of the 70 S initiation complex formed after separation from its reaction components by agarose 1.5m gel filtration. The results presented in Fig. 6 show that virtually no \( \alpha^3P \) associated with \( f^3H \)Met-tRNA bound to the ribosomes eluted in the void volume (Fig. 6A). However, a substantial amount of the IF-2 activity added to the initiation reaction mixture was associated with the 70 S initiation complex in the excluded volume. The latter was carried out by running an identical parallel experiment with \( f^3C \)Met-tRNA and unlabeled GTP (Fig. 6B). Free IF-2 eluted from this column in the included volume.

Further proof that most of the IF-2 and fMet-tRNA found in the region of the isolated complex was part of the 70 S initiation complex was obtained. More than 90% of the fMet-tRNA which eluted in the void volume in Fig. 6A was puromycin...
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**DISCUSSION**

To date the only specific role assigned to IF-1 is its ability to release IF-2 from ribosomes by IF-1. A large scale reaction mixture (1 ml) of bound IF-2, formed in the absence of IF-1, while the second stage involved release of IF-2 from ribosomes by IF-1. A large scale reaction mixture (1 ml) of bound IF-2 activity were incubated at 30°C for 10 min in the absence (A) and in the presence (B) of 100 units of IF-1. Subsequently chilled reaction mixtures were layered onto 5 to 20% sucrose gradients and centrifuged as described in the legend to Fig. 1 except that GTP was not included in the gradient. Fractions of 0.2 ml were collected, and IC radioactivity due to 14C-labeled IF-2 activity was determined by counting aliquots in Bray's solution in a scintillation counter. Gradient fractions were also assayed for IF-2 activity as described in legend to Fig. 1.

![Fig. 6. Attempts to detect the presence of GDP in isolated 70 S initiation complex.](http://www.jbc.org/)

The experiment was carried out in two stages. The first stage involved isolation of a 70 S initiation complex containing bound IF-2, formed in the absence of IF-1, while the second stage involved release of IF-2 from ribosomes by IF-1. A large scale reaction mixture (1 ml) of bound IF-2 activity were incubated at 30°C for 10 min in the absence (A) and in the presence (B) of 100 units of IF-1. Subsequently chilled reaction mixtures were layered onto 5 to 20% sucrose gradients and centrifuged as described in the legend to Fig. 1 except that GTP was not included in the gradient. Fractions of 0.2 ml were collected, and IC radioactivity due to 14C-labeled IF-2 activity was determined by counting aliquots in Bray's solution in a scintillation counter. Gradient fractions were also assayed for IF-2 activity as described in legend to Fig. 1.

![Fig. 5. The release of IF-2 from isolated 70 S initiation complexes.](http://www.jbc.org/)

reactive while the IF-2 activity eluting in the similar position in Fig. 6 co-sedimented with the 70 S initiation complex after sucrose gradient centrifugation (data not shown). It should be noted that these experiments were done under conditions in which it can be demonstrated by the use of [γ-32P]GTP that an amount of GTP, in a stoichiometric ratio to Met-tRNA bound on the ribosome, can be hydrolyzed to GDP if IF-1 is present (data not shown). Thus if the [α-32P]GDP formed during the 70 S initiation complex formation were bound to IF-2 as a stable [IF-2-GDP] complex it would have been detected in the void volume of the 70 S complex following agarose gel filtration. We also failed to detect the presence of an [IF-2-GDP] complex bound to 70 S initiation complexes when analyzed by Millipore filtration (data not shown).

These results demonstrate that it is unlikely that a stable [IF-2-GDP] complex was formed, and even less likely that IF-2 was released as an [IF-2-GDP] complex.

**REFERENCES**

1. Thach et al. (23) have observed that IF-1 binds to the 30 S subunit during initiation complex formation. However, the precise function of IF-1 in the formation of 30 S initiation complexes remains unclear. Results presented in this communication clearly demonstrate that at the level of 30 S initiation complex formation, IF-1 has no effect on either the initial binding of IF-2, nor does it increase the amounts of IF-2-dependent fMet-tRNA and GTP bound to the 30 S subunits. The effect of IF-1 is to specifically prevent the dissociation of IF-2 from 30 S complexes in a manner independent of IF-3. The 50 S subunits also dissociate the 30 S complex is necessary for the subsequent transformation of the complex into a 70 S complex active in peptidyl transfer. It has been previously demonstrated that the hydrolysis of GTP was prevented by the use of the nonhydrolyzable analogue of GTP, 5'-guanylyl...
methylene diphosphonate, in an initiation reaction, the 70 S complexes formed were totally inert and unable to react with puromycin (9, 16, 17). GTP is normally removed via hydrolysis upon the junction of the 50 S subunit and this removal of the nucleotide alters the conformation of the complex to place fMet-tRNA in a reactive site. Guanylyl methylene diphosphonate-like intact GTP can be removed from 30 S complexes by suitable phosphate cannot be removed by the normal hydrolytic event and junction with the 50 S subunit yields an inactive 70 S complex. However, guanylyl methylene diphosphonate-like intact GTP can be removed from 30 S complexes by suitable manipulation of the in vivo

situation the only means available for the removal of GTP does not occur, but GTP hydrolysis is obligatory to release GTP from the ribosome and allow correct positioning of fMet-tRNA for subsequent peptidyl transfer reactions.

Experiments presented in Fig. 4 and Table I demonstrate that 30 S complexes formed in the absence of IF-1 and deliberately depleted of their GTP and IF-2 content, can still accept 50 S subunits to form 70 S complexes capable of donating fMet-tRNA into peptide linkage (4). Therefore a "translocation" of fMet-tRNA dependent on the free energy of hydrolysis of GTP does not occur, but GTP hydrolysis is obligatory to release GTP from the ribosome and allow correct positioning of fMet-tRNA for subsequent peptidyl transfer reactions.

As reported previously, IF-1 is able to catalyze the release of IF-2 from ribosomes following 70 S initiation complex formation providing GTP hydrolysis has occurred (9, 10, 13, 16, 17, 24). Benne et al. (9, 12) have suggested that IF-1 may act in a manner analogous to EF-T, (18) and they have proposed the following mechanism:

\[
\text{[IF-2-GTP] + GDP} \xrightarrow{+ \text{IF-1}} \text{[IF-2-GTP] + GDP} \xrightarrow{[IF-2-GTP]} \text{IF-2 + GDP}
\]

where IF-1 mediates an exchange reaction between GTP and GDP for IF-2 release. This proposal is based upon the assumption that an [IF-2-GDP] complex exists on the ribosome. Results presented in this communication clearly show that a stable complex is not formed between IF-2 and GDP and that 70 S ribosomal complexes containing IF-2 and lacking GDP can be isolated by gel filtration (see Fig. 6). Therefore the possibility that IF-1 may act by destabilizing an [IF-2-GDP] complex or that an exchange between GDP and GTP occurs can be ruled out.

The results presented in Fig. 5 show that when 70 S initiation complexes are isolated free from all unreacted reaction components the only factor necessary for the release of IF-2 is IF-1. Since addition of GTP is not necessary for release of IF-2, a reaction between bound IF-2 and GTP mediated by IF-1 can also be ruled out.

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Function of initiation factor 1 in the binding and release of initiation factor 2 from ribosomal initiation complexes in Escherichia coli.

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Additions and Corrections

Vol. 252 (1977) 1739-1744

Function of initiation factor 1 in the binding and release of initiation factor 2 from ribosomal initiation complexes in Escherichia coli.

Evan A. Stringer, Probir Sarkar, and Umadas Maitra

Page 1741, Legend to Fig. 2, line 3

No IF-1 was added in these experiments. The line should read:

"Two initiation reaction mixtures, A and B (0.2 ml each) were prepared as described in legend to Fig. 1 except that 60 units of IF-2, 30 units of IF-3, and 2.4 A_{mO} units of 30 S subunit were added to each reaction mixture."

Vol. 252 (1977) 6169-6176

Induction of different rat liver supernatant aldehyde dehydrogenases by phenobarbital and tetrachlorodibenzo-p-dioxin.

Richard A. Deitrich, Pequita Bludeau, Thomas Stock, and Michael Roper

Page 6169, Paragraph 3, line 14

"Y enzyme should be T enzyme. The lines should read:"

Vol. 252 (1977) 3525-3532

Characterization of polypeptides associated with messenger RNA and its polyadenylate segment in Ehrlich ascites messenger ribonucleoprotein.

William R. Jeffery

Pages 3528 and 3529, Figs. 3 and 5

Photograph of gels shown in Fig. 3 is actually Fig. 5, while gels shown in Fig. 5 are actually those of Fig. 3.

Page 3530

In the last paragraph before "Discussion," references to Fig. 6, F and F are interchanged. Thus, line 11 from the end of the paragraph should read

"Photographs. As shown in Fig. 6 F, the salt wash proteins extract..." Line 6 from the end of the paragraph should read

"from oligo(dT)-cellulose mixed with salt wash alone (Fig. 6E)"

Vol. 252 (1977) 6342-6348

Cyclic AMP-binding proteins and protein kinase during regression of Walker 256 mammary carcinoma.

Yoon Sang Cho-Chung, Timothy Clair, and Rochelle Porper

Page 6345, Fig. 4

Due to a printer's error in the final photographing stage, part of Fig. 4 was covered by type that had slipped. The correct figure appears below.

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Additions and Corrections

Vol. 252 (1977) 1739-1744

Function of initiation factor 1 in the binding and release of initiation factor 2 from ribosomal initiation complexes in Escherichia coli.

Evan A. Stringer, Probir Sarkar, and Umadas Maitra

Page 1741, Legend to Fig. 2, line 3

No IF-1 was added in these experiments. The line should read:

Two initiation reaction mixtures, A and B (0.2 ml each) were prepared as described in legend to Fig. 1 except that 60 units of IF-2, 30 units of IF-3, and 2.4 A\textsubscript{100} units of 30 S subunit were added to each reaction mixture.

Vol. 252 (1977) 3525-3532

Characterization of polypeptides associated with messenger RNA and its polyadenylate segment in Ehrlich ascites messenger ribonucleoprotein.

William R. Jeffery

Pages 3528 and 3529, Figs. 3 and 5

Photograph of gels shown in Fig. 3 is actually Fig. 5, while gels shown in Fig. 5 are actually those of Fig. 3.

Page 3530

In the last paragraph before "Discussion," references to Fig. 6, E and F are interchanged. Thus, line 11 from the end of the paragraph should read

phosphorylation. As shown in Fig. 6 F, the salt wash proteins ex-

Line 6 from the end of the paragraph should read

from oligo(dT)-cellulose mixed with salt wash alone (Fig. 6E)

Vol. 252 (1977) 6169-6176

Induction of different rat liver supernatant aldehyde dehydrogenases by phenobarbital and tetrachlorodibenzo-p-dioxin.

Richard A. Deitrich, Pequita Bludeau, Thomas Stock, and Michael Roper

Page 6169, Paragraph 3, line 14

Y enzyme should be T enzyme. The lines should read:

the enzyme occurs. (d) The \( \phi \) enzyme has a \( K_m \) for acetaldehyde at pH 7.4 of about 0.22 mM, while the T enzyme has a \( K_m \) of about 2.6 mM. (e) The molecular weight as determined by

Vol. 252 (1977) 6342-6348

Cyclic AMP-binding proteins and protein kinase during regression of Walker 256 mammary carcinoma.

Yoon Sang Cho-Chung, Timothy Clair, and Rochelle Porper

Page 6345, Fig. 4

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