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Regulation of protein synthesis in rabbit reticulocyte lysates by the heme-regulated protein kinase: Inhibition of interaction of Met-tRNA$_f^{Met}$ binding factor with another initiation factor in formation of Met-tRNA$_f^{Met}$·40S ribosomal subunit complexes

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Regulation of protein synthesis in rabbit reticulocyte lysates by the heme-regulated protein kinase: Inhibition of interaction of Met-tRNA$_f^{Met}$ binding factor with another initiation factor in formation of Met-tRNA$_f^{Met}$·40S ribosomal subunit complexes

(phosphorylation of Met-tRNA$_f^{Met}$ binding factor/translational regulation/inhibition of Met-tRNA$_f^{Met}$ binding/40S ribosomal subunits/protein–protein interaction)

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ABSTRACT **Protein synthesis in reticulocytes and their lysates is regulated by heme. In heme deficiency a heme-regulated translational inhibitor (HRI) that blocks initiation of polypeptide chains is activated. HRI is a protein kinase (ATP: protein phosphotransferase, EC 2.7.1.37) that specifically phosphorylates the 38,000-dalton subunit of the Met-tRNA$_f^{Met}$ binding factor (IF), which forms a ternary complex with Met-tRNA$_f^{Met}$ and GTP, a finding that suggests that the inhibition by HRI involves the phosphorylation of IF.**

We have investigated the effect of HRI in the partial reactions of protein chain initiation in which the IF-promoted binding of Met-tRNA$_f^{Met}$ to 40S ribosomal subunits is enhanced by another initiation factor [ternary complex dissociation factor (TDF)] and AUG. The results show that HRI at very low concentrations markedly inhibits the binding of Met-tRNA$_f^{Met}$ to 40S subunits. The inhibitory effect of HRI requires ATP. Under these conditions HRI phosphorylates only the 38,000-dalton subunit of IF.

The TDF preparations not only promote the binding of the ternary complex to 40S subunits but also promote the dissociation of the ternary complex in the presence of 5 mM Mg^{2+} at 0°. The preincubation of purified IF alone with low concentrations of HRI and ATP does not significantly affect its capacity to form the ternary complex; however, the TDF-promoted dissociation of the ternary complex is inhibited. The nonhydrolyzable analog adenosine 5′-[β,γ-imido]triphosphate does not substitute for ATP. These findings suggest that phosphorylation causes a conformational modification in IF, which results in inhibition of the interaction between the ternary complex and TDF that is required for the binding of the ternary complex to 40S subunits.

Protein synthesis in rabbit reticulocytes and their lysates is regulated by heme (1–3). In heme deficiency, a heme-regulated translational inhibitor (HRI) is activated (4–6), which blocks protein chain initiation (2, 3, 5–10). The inhibition of protein synthesis is associated with the disappearance of the complexes of Met-tRNA$_f^{Met}$ (Met-tRNA$_f$) and 40S ribosomal subunits (9). The purified HRI has functional properties that are similar to those of the *in situ* heme-regulated inhibitor (11, 12). The inhibition induced in heme deficiency or by the addition of HRI is overcome or prevented by the Met-tRNA$_f$ binding factor (11, 13–15), which forms a ternary complex with Met-tRNA$_f$ and GTP (16–22). The Met-tRNA$_f$ binding factor (IF) is called EIF-1 in the nomenclature of Gupta and coworkers (23) and eIF-2 in the proposed new nomenclature (24); in this paper, it is called IF. The HRI has been shown to be an adenosine 3′:5′-cyclic monophosphate (cAMP) independent protein kinase (ATP: protein phosphotransferase, EC 2.7.1.37), which specifically phosphorylates the 38,000-dalton subunit and not the 52,000-dalton and 50,000-dalton subunits of IF (12, 25–28). These findings suggest that the inhibition by HRI involves the phosphorylation of IF. Significant inhibition of the binding of Met-tRNA$_f$ to 40S subunits has been shown to occur with high concentrations of HRI (13, 29, 30), and some inhibition of ternary complex formation has also been reported (31). However, the question of the molecular mechanism by which HRI exerts its inhibitory effect is not fully resolved.

In the present studies we have investigated the effect of HRI in the partial reactions of protein chain initiation in which the IF-promoted binding of Met-tRNA$_f$ to 40S subunits is enhanced by another initiation factor [ternary complex dissociation factor (TDF) of Majumdar *et al.* (23)] and AUG. The results show that HRI at very low concentrations markedly inhibits the binding of Met-tRNA$_f$ to 40S subunits. This inhibitory effect of HRI requires ATP. Under these conditions, HRI phosphorylates only the 38,000-dalton subunit of IF.

The TDF preparations not only promote the binding of the ternary complex to 40S subunits but also dissociate the ternary complex in the presence of 5 mM Mg^{2+} at 0° (23). At low concentrations of HRI and in the presence of ATP, this TDF-promoted dissociation of the ternary complex is inhibited. These results are consistent with the view that the phosphorylation of IF by HRI modifies its conformation and thus inhibits the protein–protein interaction of IF with one or more components of TDF in the formation of the Met-tRNA$_f$·40S subunit initiation complex.

Recently Das and Gupta (32) reported the isolation of a ternary complex dissociation inhibitor (TDI) from reticulocyte ribosome-free supernate. Like HRI, TDI inhibits ternary complex dissociation and the binding of Met-tRNA$_f$ to 40S subunits. In our experiments TDI and purified HRI behave similarly in all the results presented here. The apparent lack of requirement of ATP for TDI activity previously reported (32) was probably due to the presence of ATP in crude preparations of TDI.

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Abbreviations: HRI, heme-regulated translational inhibitor; tRNA$_f$, tRNA$_f^{Met}$; IF, Met-tRNA$_f$ binding factor; cAMP, cyclic AMP; TDF, ternary complex dissociation factor; TDI, ternary complex dissociation inhibitor.

MATERIALS AND METHODS

Materials. [^{35}S]Methionine (300–500 Ci/mmol) was purchased from Amersham/Searle (diluted with unlabeled methionine to give 10,000–20,000 cpm/pmol). The sources of most of the other materials utilized in these studies have been described (11, 12).

Preparation of Rabbit Reticulocyte Lysates and Assay of Protein Synthesis. The procedure for the preparation of reticulocytes, reticulocyte lysates, and the incubation mixtures for protein synthesis have been described (11, 12, 15). Protein synthesis was assayed by the incorporation of [^{14}C]leucine (0.4 μCi, specific activity 150 mCi/mmol) into proteins in 30-μl aliquots and in the presence of 10 μM hemin. Incubation was at 30°.

Preparation of HRI and TDI. HRI was prepared with some modifications of the method described before (12). Briefly, the procedure involves ammonium sulfate precipitation (0–40% saturation) and chromatography on DEAE-cellulose and phosphocellulose (at pH 6.7). These steps remove most of the cAMP-dependent protein kinase (25), but the HRI preparation still contains some protein kinase activity that phosphorylates the 50,000-dalton subunit of IF. This latter protein kinase activity is removed by chromatography on another phosphocellulose column equilibrated with Tris·HCl, 50 mM (pH 7.7)/KCl, 40 mM/dithiothreitol, 0.25 mM. Under these conditions most of the HRI cAMP-independent protein kinase does not bind to phosphocellulose (12). The protein in the eluate is bound to a DEAE-cellulose (0.5 × 7 cm) column equilibrated with Tris·HCl, 20 mM (pH 7.7)/KCl, 100 mM/10% (vol/vol) glycerol (buffer A). The residual cAMP-dependent protein kinases are removed by elution with 20 μM cAMP in buffer A. The column is washed free of cAMP, and HRI is eluted with buffer A containing 250 mM KCl. The HRI preparation obtained phosphorylates only the 38,000-dalton subunit of IF. The preparation of TDI has been described (32). Because the activities of TDI appear to be the same as those of the more highly purified HRI, we have used HRI for the studies reported here.

Preparation of IF, TDF, and 40S Ribosomal Subunits. The IF was purified from 0.5 M KCl ribosomal salt wash by a procedure modified from Ranu and Wool (20). One microgram of purified IF bound 3 pmol of Met-tRNA$_f$ in ternary complex. This preparation of IF contains low levels of TDF activity and a low level of the protein kinase activity that phosphorylates the 50,000-dalton subunit; it has no detectable protein kinase activity for the 38,000-dalton subunit. The preparation of IF free of TDF has been described (33); 1 μg of this factor preparation bound 1.2 pmol of Met-tRNA$_f$ in ternary complex. The preparation of TDF and of 40S subunits was as described previously (23, 34).

In some experiments a pooled DEAE-cellulose-chromatographed fraction of initiation factors was used (34). This initiation factor preparation, which contains IF and TDF activities, is referred to as Fraction II (34).

Assay of the HRI- and ATP-Dependent Inhibition of the TDF-Promoted Dissociation of Ternary Complex. TDF extensively promotes the dissociation of ternary complex at 0° in the presence of 5 mM Mg^{2+} (23). The inhibition assay was carried out in two stages. The first stage involves the incubation of purified IF (free of TDF activity) with HRI and ATP (phosphorylation stage). In the second stage ternary complex is allowed to form in the presence of TDF, GTP, and Met-tRNA$_f$, and the inhibition of the dissociation reaction is assayed, after incubation at 0° for 15 min in the presence or absence of 5 mM Mg^{2+}. The complete reaction mixture (50 μl) in the first stage contained Tris·HCl (pH 8.0), 30 mM; KCl, 120 mM; dithiothreitol, 3 mM; magnesium acetate, 1.3 mM; ATP, 0.2 mM; 10 μg of bovine serum albumin; 5 μg of IF; and various concentrations of HRI. Incubation was at 37° for 5 min. Samples were transferred to an ice bath and 5 μg of TDF, 0.2 mM GTP, and 8–10 pmol of [^{35}S]Met-tRNA$_f$ (10,000 cpm/pmol) were added. The final volume in the second stage was 75 μl, and the concentrations of buffer components were Tris·HCl (now pH 7.5), 20 mM; KCl, 80 mM; magnesium acetate, 1 mM; and dithiothreitol, 2 mM. Samples were incubated at 37° for 5 min and transferred to an ice bath. Magnesium acetate (5 mM) was added to the samples. In the controls, addition of magnesium was omitted. After 15 min in the ice bath, the amount of ternary complex was assayed by Millipore filtration (16–23).

Table 1. Effect of HRI, ATP, and TDF on IF-dependent formation of ternary complex (IF·GTP·Met-tRNA$_f$)

Additions	Ternary complex, pmol −ATP	Ternary complex, pmol +ATP
—	0.03	0.03
IF	4.3	4.3
HRI	0.05	0.05
TDF	0.15	0.15
IF + HRI	4.5	4.2
IF + TDF	3.9	3.95

The assay was carried out in two stages essentially as described for the TDF-promoted dissociation of the ternary complex in *Materials and Methods*. The magnesium concentration during the second stage of ternary complex formation and during 15 min of incubation at 0° was 1 mM. The protein concentrations of IF, HRI, and TDF used in the assay were 5 μg, 0.15 μg, and 5 μg, respectively; IF bound 6.25 pmol of Met-tRNA$_f$ in ternary complex in the absence of Mg^{2+}.

Assay of the Binding of Met-tRNA$_f$ to 40S Ribosomal Subunit. The binding of Met-tRNA$_f$ to 40S subunits was assayed by the two-stage assay system on Millipore filters according to Chatterjee *et al.* (34) or by sucrose density gradient centrifugation (34). The reaction mixture (20 μl) in the first stage contained Tris·HCl (pH 8.0), 30 mM; KCl, 120 mM; dithiothreitol, 3 mM; magnesium acetate, 3 mM; bovine serum albumin, 4 μg; IF, 0.8 μg; and TDF, 2 μg. Samples were incubated at 37° for 5 min with or without 0.2 mM ATP and various concentrations of HRI. The reaction mixture was transferred to an ice bath, and GTP, 0.2 mM; 40S subunits, 0.04 A_{260} unit; AUG, 0.008 A_{260} unit; and 4–5 pmol of [^{35}S]Met-tRNA$_f$ were added (an A unit is the amount of material having an absorbance of 1 when dissolved in 1 ml and the light path is 1 cm). In controls either AUG or 40S subunits were omitted. The volume of reaction mixture at this stage was 30 μl, and the final concentrations of the buffer components were Tris·HCl (now pH 7.5), 20 mM; KCl, 80 mM; magnesium acetate, 2 mM; and dithiothreitol, 2 mM. The samples were incubated at 37° for 5 min and transferred to an ice bath, and magnesium acetate, 5 mM, was added. The solution was filtered through a Millipore. The filters were washed three times with 2 ml of buffer. The details of the sucrose density gradient method have been described (32, 34).

RESULTS

The results in Table 1 show that the preincubation of purified IF with HRI or ATP or TDF or in the presence of HRI and ATP does not appreciably influence the amount of ternary complex formed when the Mg^{2+} concentration is maintained at 1 mM during the assay.

If the concentration of Mg^{2+} is raised to 5 mM during the 15 min of incubation at 0°, TDF promotes the dissociation of the

Table 2. Inhibition of TDF-promoted dissociation of ternary complex (IF·Met-tRNA$_f$·GTP) by HRI

TDF, μl	HRI, μl	Ternary complex, pmol −ATP	Ternary complex, pmol +ATP
—	—	3.87	3.32
10	—	1.27	0.96
10	1	1.20	5.05
10	5	1.27	5.31
10	10	1.27	5.37

The TDF-promoted dissociation of ternary complex was assayed as described in *Materials and Methods*. Protein concentrations of TDF and HRI preparations used were 0.5 and 0.15 mg/ml, respectively. In the absence of Mg^{2+} the Met-tRNA$_f$ binding factor (5 μg) used bound 6.25 pmol of Met-tRNA$_f$ in ternary complex.

ternary complex in the presence or absence of HRI or of ATP (Table 2). However, the ternary complex formed with Met-tRNA$_f$ binding factor preincubated with HRI and ATP is not dissociated (Table 2). The nonhydrolyzable analog adenosine 5′-[β,γ-imido]triphosphate does not substitute for ATP (results not shown).

The preparation of partially purified initiation factors (Fraction II) promotes binding of Met-tRNA$_f$ to 40S ribosomal subunits, which is enhanced and stabilized by AUG (Table 3). HRI in low concentration markedly (70–80%) inhibits the binding of Met-tRNA$_f$ to 40S subunits (Table 3). The inhibitory effect of HRI requires ATP, and both the AUG-independent and the AUG-dependent binding of Met-tRNA$_f$ are inhibited. The inhibition of Met-tRNA$_f$ binding to 40S subunits that is observed by the Millipore filtration assay can also be demonstrated by the sucrose density gradient centrifugation (Fig. 1). Once again adenosine 5′-[β,γ-imido]triphosphate does not substitute for ATP (results not shown). For an explanation of the −AUG, −40S subunit column in Table 3 see ref. 34.

The inhibitory effect of HRI, which requires ATP, could also be demonstrated in a fractionated system in which the binding of Met-tRNA$_f$ to 40S subunits is promoted by purified IF, TDF, and AUG (Table 4).

Fig. 2 shows that the HRI concentrations that inhibit the reaction of binding Met-tRNA$_f$ to 40S subunits (Table 3 and 4) also inhibit protein synthesis in the lysate system, essentially as observed in heme deficiency.

The data in Fig. 3 show that, under the conditions of inhibition of Met-tRNA$_f$ binding to 40S subunits (Tables 3 and 4), HRI phosphorylates the 38,000-dalton subunit of the Met-tRNA$_f$ binding factor. The other polypeptides of either the 40S ribosomal subunit or of the TDF preparation are not phosphorylated. The TDF preparation, however, contains protein kinase(s) that phosphorylate four polypeptides of about 60,000, 70,000, 74,000 and 84,000 daltons, respectively. The intensity of phosphorylation of the 60,000-dalton polypeptide is demonstrably higher in all the preparations of TDF that we have examined for this autophosphorylation reaction.

Table 3. Inhibition by HRI of Met-tRNA$_f$ binding to 40S ribosomal subunits with the use of partially purified initiation factors (Fraction II)

Preincubation of Fraction II with	Met-tRNA$_f$ bound, pmol −AUG −40S subunits	−AUG +40S subunits	+AUG +40S subunits
—	0.29	0.32	2.22
ATP (0.13 mM)	0.87	0.42	2.20
HRI	0.78	0.63	2.61
HRI + ATP (0.13 mM)	0.56	0.17	0.87

Fraction II (20 μg) was incubated in 50 μl of reaction mixture in stage I with the indicated components for 5 min at 37° and transferred to an ice bath. HRI concentration was 0.15 μg/50 μl. GTP, 0.2 mM; 40S subunits, 0.08 A_{260} unit; AUG, 0.02 A_{260} unit; and 8–10 pmol of [^{35}S]Met-tRNA$_f$ were added (reaction mixture 75 μl). After 5 min at 37°, the samples were transferred to an ice bath and the Mg^{2+} concentration was increased to 5 mM. After 15 min the amount of Met-tRNA$_f$ bound was assayed by Millipore filtration (23).

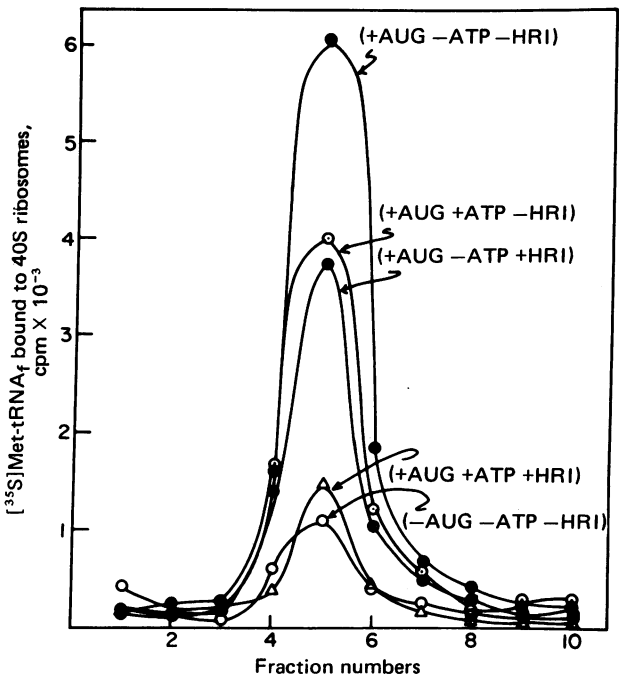

FIG. 1. Inhibition of Met-tRNA$_f$ binding to 40S subunits by HRI: assay by sucrose density gradient centrifugation. The assay conditions were as described for the experiment in Table 3 and the details of the sucrose gradient centrifugation have been described (32).

DISCUSSION

HRI markedly inhibits the binding of Met-tRNA$_f$ to 40S subunits, and the inhibition requires ATP. The physiologic nature of the inhibition is supported by the fact that low concentrations of HRI that inhibit the binding reaction also inhibit protein synthesis in the lysate system to approximately the same extent as in heme deficiency. Previously, it was shown that one inhibitory unit of HRI (9×10^9 molecules) inactivates approximately 7.5×10^{12} molecules of IF in 50 μl of the lysate reaction mixture (12, 35); on the basis of these data, there are 5.4×10^9 molecules of HRI, which inhibit 4.5×10^{12} molecules of IF in

Table 4. Inhibition by HRI of Met-tRNA$_f$ binding to 40S ribosomal subunits with the use of purified IF and a preparation of TDF

Preincubation of IF and TDF with HRI, μg	ATP	Met-tRNA$_f$ bound, pmol −AUG +40S subunits	+AUG +40S subunits
—	−	0.25	1.10
—	+	0.24	1.02
0.05	−	0.27	1.06
0.05	+	0.22	0.69
0.15	−	0.26	1.03
0.15	+	0.22	0.35

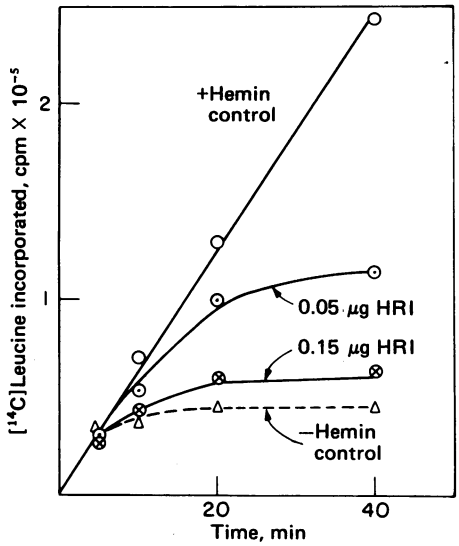

FIG. 2. Inhibition of protein synthesis by HRI. Reaction mixtures (30 μl) containing 10 μM hemin were incubated at 30° with indicated amounts of HRI. Controls without hemin and with hemin but without HRI were also incubated. At intervals, 5-μl aliquots were removed and protein synthesis was assayed.

a 30-μl protein synthesis reaction mixture (Fig. 2). This number corresponds approximately to the 3.4×10^{12} molecules of IF in the reaction mixture (Table 4) used in the inhibition of Met-tRNA$_f$ binding. The degree of inhibition of Met-tRNA$_f$ binding and the requirements of the inhibition reaction that we have observed with the fractionated system are in agreement with similar results obtained by Farrell *et al.* (27) in experiments in which crude ribosomes with the full complement of initiation factors from lysates passed through Sepharose 6B were used.

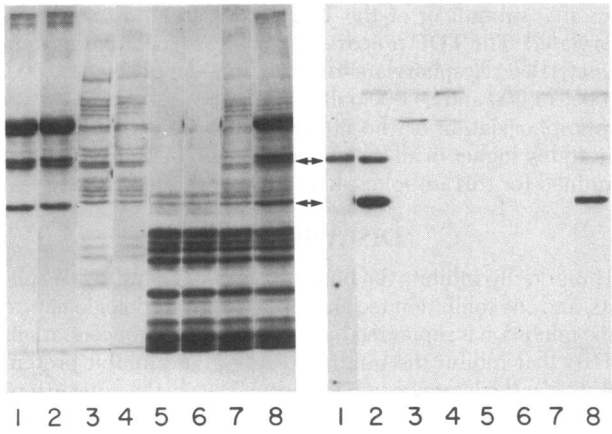

FIG. 3. Phosphorylation of initiation factors. Reaction mixtures (15 μl) in buffer [Tris·HCl, 20 mM (pH 7.5)/KCl, 80 mM/magnesium acetate, 2 mM/dithiothreitol, 0.5 mM] containing 0.2 mM [γ-^{32}P]ATP (2000 cpm/pmol) were incubated at 30° for 15 min with: track 1, IF (0.8 μg); track 2, IF (0.8 μg) and HRI (0.15 μg); track 3, TDF (2 μg); track 4, TDF (1 μg) and HRI (0.15 μg); track 5, 40S subunits (0.08 A_{260} unit); track 6, 40S subunits (0.08 A_{260} unit) and HRI (0.15 μg); track 7, TDF (1 μg), 40S subunits (0.08 A_{260} unit), and HRI (0.15 μg); and track 8, TDF (1 μg), IF (0.8 μg), 40S subunits (0.08 A_{260} unit), and HRI (0.15 μg). Reaction was terminated by the addition of 15 μl of denaturing solution [Tris·HCl, 50 mM (pH 7.0)/2% sodium dodecyl sulfate/5% (vol/vol) 2-mercaptoethanol]. Samples were heated at 100° for 2 min and electrophoresed in sodium dodecyl sulfate/polyacrylamide (10%) gel at 140 V for 2.5 hr (12). The gels were stained with Coomassie brilliant blue (*Left*) and autoradiograms were prepared (*Right*) (12). Migration is toward the bottom. Arrows indicate the position of 50,000- and 38,000-dalton subunits of IF. The HRI preparation contains an autophosphorylating protein that migrates at 80,000 daltons (tracks 2, 4, 6, 7, and 8). Note that bovine serum albumin (69,000 daltons) was added to the IF preparation.

We have, however, observed that with high concentrations of HRI (3–4 times the concentrations used in the data presented here) there is significant but variable inhibition of Met-tRNA$_f$ binding to 40S subunits. Because there is no requirement for ATP for the inhibition, the biological significance of these results is doubtful. The inhibition of Met-tRNA$_f$ binding to 40S subunits with crude preparations of HRI and initiation factors has been reported (13); it is probable that the observed inhibition was due to the presence of ATP in the preparations. Previously, inhibition of Met-tRNA$_f$ binding to 40S subunits with the use of a homogeneous preparation of IF was reported (15); but subsequently, the possibility was raised that one or more additional factors might be required (36). Inhibition of Met-tRNA$_f$ binding to 40S subunits with prephosphorylated IF (with HRI) and another initiation factor has also been reported by Kramer *et al.* (30), who suggested that the inhibition of protein–protein interaction with components of the 40S subunit·Met-tRNA$_f$ initiation complex may be involved.

Darnbrough *et al.* (37) have shown that there is a rapid exchange of 40S subunit-bound Met-tRNA$_f$ with free Met-tRNA$_f$. They suggested that, in the absence of mRNA, the 40S subunit-bound ternary complex may undergo very rapid dissociation and association reactions that are in equilibrium, and that AUG (or mRNA) stabilizes the bound ternary complex and shifts the equilibrium to the stable 40S·Met-tRNA$_f$·AUG complexes. Such a mechanism is compatible with the ternary complex dissociation activity that is associated with TDF and with the marked enhancement of Met-tRNA$_f$ binding to the 40S subunit by AUG under physiological conditions (23, 34). Because the binding of the ternary complex to the 40S subunit requires TDF, and dissociation of the ternary complex also requires TDF, and because both of these functions are inhibited by HRI, there may be a functional relationship, still undetermined, between ternary complex dissociation and Met-tRNA$_f$ binding to the 40S subunit.

The marked inhibition of Met-tRNA$_f$ binding to 40S subunits might appear to be incompatible with the inhibition of recycling of IF by phosphorylation (35). However, the finding that there is a period of protein synthesis at the control rate before the onset of inhibition in heme deficiency or in the presence of activated HRI, and the presence of a pool of IF (35) in lysates, suggest that a significant portion of the pool may not be accessible to phosphorylation by HRI, perhaps because IF is bound to 40S subunits or is in a complex with other initiation factors. After the factor has participated in one cycle of protein chain initiation it might become susceptible to inactivation by phosphorylation. Such a mechanism could account for the constant level of protein synthesis before shutoff that has been observed in heme deficiency (6) and in the presence of HRI (11). When HRI is added to a lysate whose pool of IF is depleted, the onset of inhibition is very rapid (11), a finding consistent with the marked inhibition by HRI of the formation of Met-tRNA$_f$·40S subunit complexes.

Phosphorylation of the 38,000-dalton subunit of IF is required for the inhibition of Met-tRNA$_f$ binding and of the TDF-promoted dissociation of the ternary complex. This IF preparation contains some protein kinase that phosphorylates the 50,000-dalton subunit of IF (38). However, the phosphorylation of this subunit is not required for the inhibition of protein synthesis in lysates (27), nor does it influence Met-tRNA$_f$ binding to the 40S subunit or the capacity of TDF to dissociate

the ternary complex. There are three to four polypeptides of TDF that are phosphorylated by endogenous protein kinase(s) in the TDF preparations. Whether phosphorylation of these polypeptides is required for the activity of TDF in the protein synthesis cycle and for the inhibition of partial reactions of protein chain initiation that we have described remains to be seen. The phosphorylation of several polypeptides of TDF that we have described has not previously been reported. The finding that HRI does not phosphorylate any of the approximately 30 polypeptides of 40S subunits (27) or any of the 10–14 major (23) and 6–8 minor polypeptides of TDF provides further evidence of the specificity of the HRI protein kinase. In our studies, phosphorylation of 40S subunit proteins was not required for the inhibition of the Met-tRNA$_f$ binding reaction, but the possibility that 40S subunits are already phosphorylated cannot be excluded.

Previous studies have indicated that Met-tRNA$_f$ binding to IF is controlled by two protein factors, TDF (23) and Co-EIF-1 (39), the latter of which increases the rate and extent of ternary complex formation. The results presented in this paper suggest that phosphorylated IF is not recognized by TDF, an interaction possibly essential for Met-tRNA$_f$ binding to 40S ribosomes. With a partially purified initiation factor preparation (Fraction II), we have also observed significant inhibition (50%) of ternary complex formation (at 1 mM Mg^{2+}) by HRI and ATP (unpublished observation). These preparations contain significant amounts of Co-EIF-1 activity. A possible explanation of this inhibition of ternary complex formation by HRI and ATP is that the phosphorylated IF is not recognized by Co-EIF-1. Inhibition of ternary complex formation by HRI and ATP has also been reported by Datta *et al.* (31).

Note Added in Proof. Rat liver cytosol contains a translational inhibitor protein kinase (LI) which has properties similar to those of HRI (40). Like HRI, LI also inhibits the TDF-promoted dissociation of ternary complex and the binding of ternary complex to 40S subunits. These inhibitions require ATP.

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