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Mechanism of polypeptide chain initiation in eukaryotes and its control by phosphorylation of the α subunit of initiation factor 2

(translational control/eukaryotic initiation factor 2-stimulating protein/eukaryotic initiation factor 2–protein complex)

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ABSTRACT **Earlier, we isolated eukaryotic initiation factor 2 (eIF-2)-stimulating protein (SP) as a homogeneous complex with eIF-2 (eIF-2–SP) and showed that, in the presence of Mg^{2+}, eIF-2–SP promotes formation of a ternary complex with GTP and eukaryotic initiator methionyl tRNA (Met-$tRNA_i$) (eIF-2–GTP–Met-$tRNA_i$) catalytically. We now show that SP-bound eIF-2 exchanges with eIF-2 (eIF-2 exchange). Furthermore, in the presence of Mg^{2+}, eIF-2–SP catalyzes the exchange of eIF-2-bound [^{3}H]GDP with unlabeled GDP or GTP (GDP exchange) and the release of [^{3}H]GDP when the ternary complex is formed from eIF-2–[^{3}H]GDP, GTP, and [^{35}S]Met-$tRNA_i$. All these reactions are blocked by α-subunit, but not by β-subunit, phosphorylation of eIF-2. The eIF-2 and GDP exchanges are compatible with the reaction eIF-2–GDP + SP ⇌ EIF-2–SP + GDP reminiscent of the exchange between the Tu and Ts components of prokaryotic elongation factor 1 (EF-Tu and EF-Ts, respectively) EF-Tu–GDP + EF-Ts ⇌ EF-Tu–EF-Ts + GDP. Due to the high affinity of GDP (≈100 times greater than that of GTP) for eIF-2, 40S (eIF-2–GTP–Met-$tRNA_i$–40S) to 80S (Met-$tRNA_i$–mRNA–80S) initiation complex conversion, which is accompanied by GTP hydrolysis, probably releases eIF-2 as eIF-2–GDP. Our results suggest that, in the presence of Mg^{2+}, GDP binding restricts the availability of eIF-2 for chain initiation and that SP relieves this restriction in a catalytic fashion, provided that the α subunit of eIF-2 is not phosphorylated.**

Polypeptide chain initiation in eukaryotes begins with the formation of a ternary complex between eukaryotic initiation factor 2 (eIF-2), GTP, and eukaryotic initiation methionyl $tRNA_i$ (Met-$tRNA_i$) (eIF-2–GTP–Met-$tRNA_i$), which binds to a 40S ribosome, giving rise to a 40S initiation complex (for review, see ref. 1). At low concentrations of Met-$tRNA_i$ and eIF-2, little or no ternary complex is formed unless an additional factor is present. This factor, termed eIF-2-stimulating protein (ESP), was discovered while searching for the mechanism of translational inhibition associated with phosphorylation of the α subunit of eIF-2 (2, 3). It turned out that ESP was active with intact or β-subunit-phosphorylated but not with α-subunit-phosphorylated eIF-2 (2–4). Factors similar to ESP were later reported to relieve inhibition of ternary complex formation by Mg^{2+} (5, 6) and we confirmed this observation (7). Recently, we isolated in virtually homogeneous condition a complex of eIF-2 with ESP from the postribosomal supernatant of rabbit reticulocyte lysates (7, 8). We shall refer to this complex as eIF-2–SP, where SP stands for stimulating protein.† On electrophoresis in nondenaturing gels, the complex migrates essentially as a broad single band. In denaturing gels, it displays the α, β, and γ subunits of eIF-2 (38, 52, and 54 kilodaltons, respectively) and five SP bands (≈32, 40, 57, 65, and 80 kilodaltons) in approximately equimolar amounts. The apparent M_r of the complex is 450,000. The homogeneous factor promotes ternary complex formation catalytically in the presence of Mg^{2+} (8). Further study of the catalytic function of eIF-2–SP and its control by α-subunit phosphorylation of eIF-2 is described in this paper. Our results indicate that the mechanisms of the initial steps of eukaryotic chain initiation and both prokaryotic (9, 10) and eukaryotic (11) chain elongation are similar.

MATERIALS AND METHODS

Assays. (*i*) Ternary complex formation was measured as described (4). Samples (50 μl) of 20 mM Tris·HCl, pH 7.5/100 mM KCl/0.5 mM $Mg(OAc)_2$/2 mM 2-mercaptoethanol/22 μM GTP (GDP free) containing 5 μg of bovine serum albumin, eIF-2 (or eIF-2–[^{3}H]GDP) with or without eIF-2–SP as specified in the figures, and ^{3}H-labeled or ^{35}S-labeled Met-tRNA as specified in the figures were incubated for 6 min at 30°C. (*ii*) Binary complex formation between eIF-2 and GDP was used as a model for the physiologically relevant reaction with GTP, since reassessment of the original assay (12) showed, in agreement with a recent report (13), that only GDP forms a stable complex with eIF-2. Samples (50 μl) of 20 mM Tris·HCl, pH 7.5/100 mM KCl/2 mM 2-mercaptoethanol/2 μM [^{3}H]GDP (10,000 cpm/pmol) containing the indicated amounts of $Mg(OAc)_2$, eIF-2, and eIF-2–SP were incubated as in (*i*). (*iii*) For measurement of the exchange of GDP or GTP with eIF-2-bound [^{3}H]GDP, samples (as in *ii*) of 1–2 pmol of eIF-2–[^{3}H]GDP, the indicated amounts of eIF-2–SP, $Mg(OAc)_2$, and either GDP or GTP were incubated for 1 min at 30°C. Bound radioactivity was assayed by Millipore filtration. Samples were chilled in ice, immediately diluted with 1 ml of wash buffer (20 mM Tris·HCl, pH 7.5/100 mM KCl/1.0 mM $Mg(OAc)_2$/2.0 mM 2-mercaptoethanol), and filtered on nitrocellulose membranes. Filters were washed once with 10 ml of wash buffer. The filters were transferred to glass scintillation vials and 1 ml of methylcellosolve (Fisher) was added. The filters were dissolved with mechanical shaking and, after adding 10 ml of Hydrofluor (National Diagnostics), were assayed in a Beckman LS 100 scintillation counter. The counting

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Abbreviations: eIF-2, eukaryotic initiation factor 2; SP, eIF-2-stimulating protein; Met-$tRNA_i$, eukaryotic initiator methionyl tRNA; HCI, heme-controlled translational inhibitor (an eIF-2 α-kinase); EF-Tu and EF-Ts, components of prokaryotic elongation factor 1; EF-1α and EF-1βγ, components of eukaryotic elongation factor 1; aa-tRNA, aminoacyl-tRNA; eIF-2(αP) and eIF-2(βP), α-subunit- and β-subunit-phosphorylated eIF-2, respectively.

† Previously (8), we referred to the supernatant factor as RF-eIF-2 or simply RF (for translation restoring factor). The ribosomal salt wash factor, earlier termed ESP, was referred to as ESP–eIF-2. Both factors are in all probability one and the same protein (i.e., eIF-2–SP).

efficiency for ^3H alone was 30% and that for ^3H in the presence of ^{35}S was 23%. (*iv*) For exchange of free ^{32}P-labeled eIF-2 with SP-bound eIF-2, samples (250 μl) of 20 mM Tris·HCl, pH 7.5/100 mM KCl/0.8 mM $Mg(OAc)_2$ containing 25 μg of bovine serum albumin, 25 μg of creatine kinase, and the indicated amounts of eIF-2(β-^{32}P) at 2,800 cpm/pmol or eIF-2(α-^{32}P) at 3,700 cpm/pmol and eIF-2-SP were incubated for 5 or 2 min as indicated at 30°C in plastic tubes. Creatine kinase was added to prevent losses of eIF-2 through adsorption to the tube walls (14). After cooling in ice, 200-μl aliquots were layered onto a 4-ml 10–30% linear sucrose gradient in 20 mM Tris·HCl, pH 7.5/100 mM KCl/0.8 mM $Mg(OAc)_2$/1 mM dithiothreitol and centrifuged for 5 hr at 54,000 rpm in a Beckman SW 56 rotor. Gradients were fractionated in an Isco model 640 density gradient fractionator. Each fraction (0.2 ml) was mixed with an equal volume of water and assayed in 10 ml of Ready-Solv HP (Beckman).

Protein was determined by the Bradford (15) procedure with bovine serum albumin as the standard.

Preparations. The preparation of rabbit reticulocyte lysates and eIF-2 has been described (8). If not otherwise stated, eIF-2a [eIF-2 CM-350 (4)], ≈25% pure, and eIF-2c (16), 90% pure or greater, were used throughout. Amounts are given as pure eIF-2. The homogeneous eIF-2 used in Fig. 1 was purified by V. Manne through the last step of the procedure of Benne *et al.* (16). The preparation of homogeneous eIF-2–SP from the reticulocyte lysate postribosomal supernatant has also been described (8). eIF-2–SP has now been prepared four times with uniform results. Consistently, 800 ml of supernatant has given 0.5–0.7 mg of eIF-2–SP of specific activity (ternary complex formation assay) 40,000–60,000. The subunit structure of our most recent preparation is shown in Fig. 1. *N*-Ethylmaleimide-treated eIF-2–SP was prepared by incubating eIF-2–SP with 5 mM *N*-ethylmaleimide for 15 min at 25°C followed by neutralization of the unreacted reagent with 10 mM dithiothreitol. For preparation of eIF-2–[^3H]GDP, samples (3 ml) of 20 mM Tris·HCl, pH 7.5/100 mM KCl/2 mM 2-mercaptoethanol/20 mM [^3H]GDP (Amersham; 3,300 cpm/pmol) containing 1.18 nmol of eIF-2a were incubated for 10 min at 30°C, cooled in ice, and made 1.0 mM in $Mg(OAc)_2$. As determined by Millipore filtration, the [^3H]GDP/eIF-2 ratio (mol/mol) was 1.0. The samples were applied to a 0.7 × 2.0 cm Sepharose 6B-heparin column previously equilibrated with buffer A [20 mM Tris·HCl, pH 7.5/1.0 mM $Mg(OAc)_2$/2 mM 2-mercaptoethanol/5% (vol/vol) glycerol]/200 mM KCl. The column was washed with this buffer until all the noncomplexed [^3H]GDP was eluted; eIF-2–[^3H]GDP was then eluted with buffer A/400 mM KCl. The material (containing ≈80% pure eIF-2 as judged by $NaDodSO_4$/polyacrylamide gel electrophoresis) was stored in liquid nitrogen. For preparation of β-subunit-^{32}P-phosphorylated eIF-2 [eIF-2(β-^{32}P)], reaction mixtures (2 ml) of 10 mM Tris·HCl, pH 7.5/50 mM KCl/4 mM $Mg(OAc)_2$/1.0 mM dithiothreitol/0.1 mM [γ-^{32}P]ATP (4,000 cpm/pmol) containing 1.875 nmol of eIF-2c and 200 μg of reticulocyte casein kinase (4) were incubated for 20 min at 30°C and the phosphorylated eIF-2 was isolated by Sepharose-heparin chromatography (17) as described for the eIF-2–[^3H]GDP complex. α-Subunit-^{32}P-phosphorylated eIF-2 [eIF-2(α-^{32}P)] was similarly prepared, but in the absence of KCl, with 2 nmol of eIF-2c and 124 μg of (par-

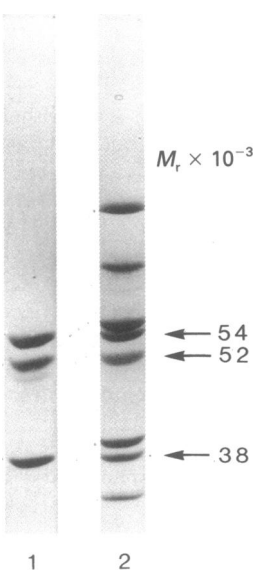

FIG. 1. $NaDodSO_4$/polyacrylamide disc gel electrophoresis of eIF-2 (lane 1) and eIF-2–SP (lane 2) (7.8 μg each). Electrophoresis [10% polyacrylamide/0.26% *N,N'*-methylenebis(acrylamide)] was for 2 hr 40 min at 6 mA per tube. Gels were stained with Coomassie blue.

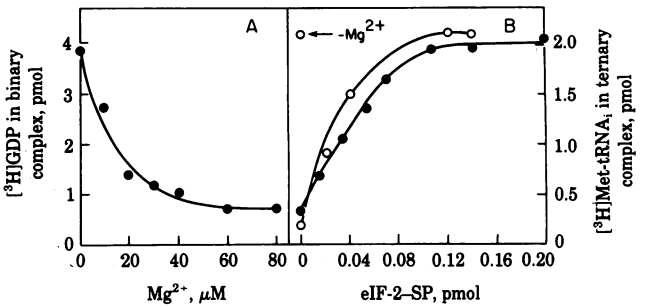

FIG. 2. (*A*) Inhibition of eIF-2–GDP binary complex formation by Mg^{2+}. Five picomoles of eIF-2a was used. (*B*) Relief of Mg^{2+} inhibition of binary (●) and ternary (○) complex formation by eIF-2–SP. Ternary complex was assayed with 5 pmol of eIF-2a and 3 pmol of [^3H]Met-tRNA$_i$ (62,000 cpm/pmol) at 0.5 mM Mg^{2+}. Binary complex was assayed at 0.1 mM Mg^{2+}. ←, Ternary complex formation in the absence of Mg^{2+} and eIF-2–SP.

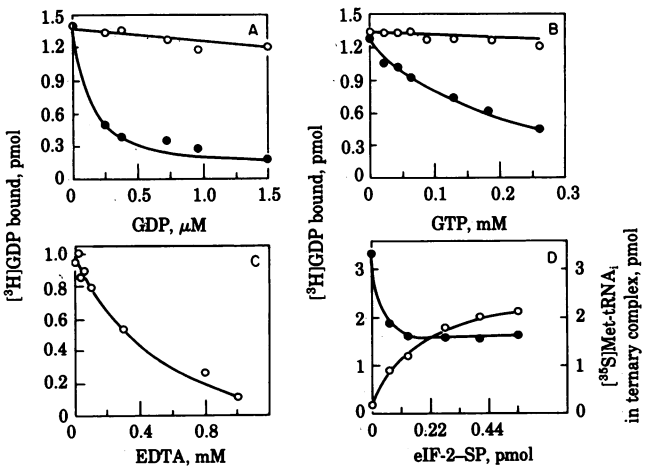

FIG. 3. Exchange of unlabeled GDP (*A*) or GTP (*B*) with eIF-2-bound [^3H]GDP as a function of the GDP (GTP) concentration in the presence of 1.0 mM Mg^{2+} and in the absence (○) or presence (●) of 0.66 pmol of eIF-2–SP (final concentration, 13 nM). (*C*) GDP exchange in the absence of eIF-2–SP on removal of Mg^{2+} by EDTA. (*D*) Release of eIF-2-bound [^3H]GDP (●) on ternary complex formation (○) at various concentrations of eIF-2–SP. This experiment was carried out three times with similar results. Ternary complex assay used 1.0 mM $Mg(OAc)_2$/22 μM GTP and 4 pmol of [^{35}S]Met-tRNA$_i$ (11,400 cpm/pmol).

tially purified) heme-controlled translational inhibitor (HCI) prepared as described (8). The phosphorylated eIF-2 preparations contained ≈1 mol of ^{32}P/mol of eIF-2 (cf. ref. 4). GDP-free GTP (used throughout) and Met-tRNA$_i$ were prepared as described (4).

RESULTS

Mechanism of Initiation. As previously reported (5–8), ternary complex formation is inhibited by Mg^{2+} and the inhibition is catalytically relieved by eIF-2–SP (7, 8). Here we show that binary complex formation between eIF-2 and GDP is similarly inhibited (Fig. 2*A*) and similarly relieved (Fig. 2*B*) by eIF-2–SP (cf. also ref. 12). The catalytic ratio (complex formed/eIF-2–SP, mol/mol), estimated from the linear portion of the curves of Fig. 2*B*, was ≈80 for binary and 40 for ternary complex formation. From the average specific activity of eIF-2–SP (50,000 pmol/mg), the catalytic ratio (for M_r 450,000) would be 22.5. This is probably a minimum value. At 1 mM Mg^{2+}, eIF-2-bound [^{3}H]GDP exchanges with unlabeled GDP (Fig. 3*A*) and, less readily, with GTP (Fig. 3*B*) in the presence, but not in the absence of eIF-2–SP unless the Mg^{2+} is chelated by EDTA (Fig. 3*C*). As previously shown (18), eIF-2 has much greater affinity for GDP than for GTP. About 1,000-fold higher concentration of GTP than GDP (at 1 mM Mg^{2+}) was required for displacement of [^{3}H]GDP from eIF-2. Nevertheless, the ternary complex is easily formed in the presence of Mg^{2+} from eIF-2–[^{3}H]GDP, GTP, and [^{35}S]Met-tRNA$_i$, provided eIF-2–SP is present (Fig. 3*D*). The functional similarity of the complex between eIF-2 and SP—i.e., eIF-2–SP—in eukaryotic chain initiation and the complexes between the two components of chain elongation factor 1—i.e., EF-Tu–EF-Ts (9, 10) and EF-1α–EF-1$\beta\gamma$ (11)—in prokaryotic and eukaryotic chain elongation is apparent. There is also an exchange of eIF-2(β-^{32}P) with eIF-2 in the eIF-2–SP complex (Fig. 4*A*). The eIF-2 exchange may be formally represented by eIF-2* + eIF-2–SP ⇌ eIF-2 + eIF-2*–SP. This exchange is very rapid (it appears to reach equilibrium in <1 min at 0°C) and occurs in the absence or presence of Mg^{2+}. Two observations suggest that the eIF-2 exchange is related to catalysis of ternary complex formation by eIF-2–SP: (*i*) the exchange is blocked by treatment of eIF-2–SP with *N*-ethylmaleimide (Fig. 4*B*), which hinders its activity in ternary complex formation (19), and (*ii*) it occurs at the optimum pH for stimulation of ternary complex formation by eIF-2–SP (Fig. 4 *C* and *D*).

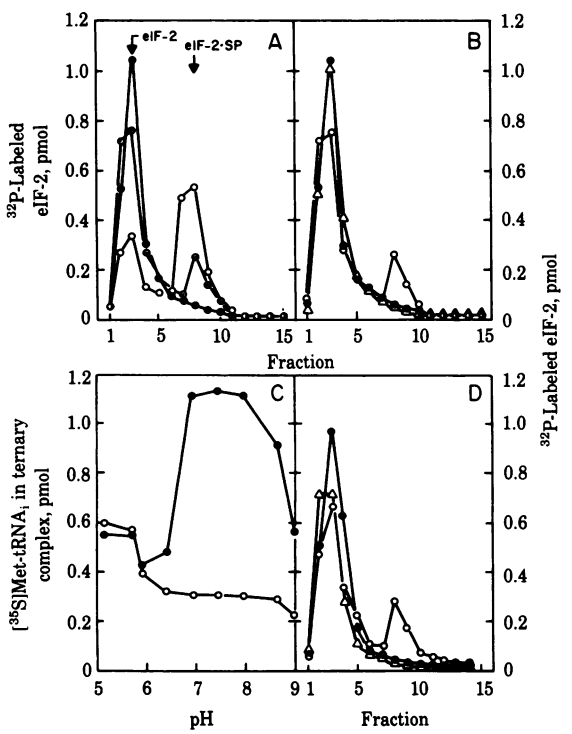

FIG. 4. Exchange of free with SP-bound eIF-2. eIF-2(β-^{32}P) was incubated with eIF-2–SP for 5 min at 30°C and the distribution of radioactivity between free and bound eIF-2 was analyzed by sucrose density gradient centrifugation. (*A*) ●, Control, eIF-2(β-^{32}P) (6.6 pmol) alone; ○, eIF-2(β-^{32}P) and eIF-2–SP (8.8 pmol); ○, eIF-2(β-^{32}P) and eIF-2–SP (13.8 pmol). (*B*) ●, Control, eIF-2(β-^{32}P) (6.6 pmol) alone; ○ eIF-2(β-^{32}P) and eIF-2–SP (8.8 pmol); △, eIF-2(β-^{32}P) and *N*-ethylmaleimide-treated eIF-2–SP (8.8 pmol). (*C*) pH dependence of eIF-2–SP-catalyzed ternary complex formation. Six picomoles of eIF-2a and 3 pmol of [^{35}S]Met-tRNA$_i$ (95,400 cpm/pmol) were used. ○, No eIF-2–SP; ●, 0.2 pmol of eIF-2–SP (final concentration, 4 nM). (*D*) Effect of pH on exchange. ●, Control, eIF-2(β-^{32}P) (6.6 pmol) at pH 7.5; ○, eIF-2(β-^{32}P) and eIF-2–SP (8.8 pmol) at pH 7.5; △, eIF-2(β-^{32}P) and EIF-2–SP (8.8 pmol) at pH 6.5.

Effect of α-Subunit Phosphorylation of eIF-2. Phosphorylation of the eIF-2 α subunit but not of the β subunit, blocks

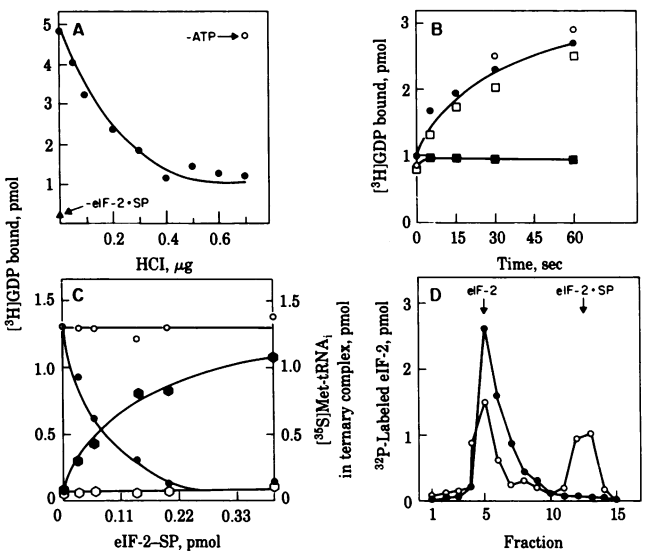

FIG. 5. (*A*) Inhibition of eIF-2–SP-stimulated eIF-2–[^{3}H]GDP binary complex formation by phosphorylation of the α subunit of eIF-2. A two-stage assay was used. (*i*) Samples (38 μl) of 20 mM Tris·HCl, pH 7.5/1.0 mM $Mg(OAc)_2$/1.0 mM dithiothreitol/0.3 mM ATP containing 5 pmol of eIF-2a and various amounts of HCI were incubated for 5 min at 30°C. (*ii*) Samples (final vol, 50 μl) were supplemented with 100 mM KCl/2 μM [^{3}H]GDP (10,000 cpm/pmol) containing 0.3 pmol of eIF-2–SP (final concentration, 6 nM), further incubated for 5 min at 30°C, and assayed for binary complex. Control reactions: ▲, Without HCI and eIF-2–SP; ○, with 0.7 μg of HCI, without ATP. (*B*) Kinetics of binary complex formation with eIF-2(αP) and eIF-2(βP). Either 5.8 pmol of eIF-2(αP) or 6.2 pmol of eIF-2(βP) was used with or without 0.3 pmol of eIF-2–SP (final concentration, 6 nM). ■, eIF-2(αP), Mg^{2+}, and eIF-2–SP; ●, eIF-2(βP), Mg^{2+}, and eIF-2–SP; □, eIF-2(αP) without Mg^{2+} or eIF-2–SP; ○, eIF-2(βP) without Mg^{2+} or eIF-2–SP. (*C*) Effect of eIF-2–SP on ternary complex formation from eIF-2–[^{3}H]GDP [or eIF-2(α-^{32}P)–[^{3}H]GDP], GTP, and [^{35}S]Met-tRNA$_i$. Release of [^{3}H]GDP and ternary complex formation with [^{35}S]Met-tRNA$_i$ were assayed simultaneously as in Fig. 3*D*. $Mg(OAc)_2$ was 1.0 mM, GTP was 65 μM, and [^{35}S]Met-tRNA$_i$ (8,400 cpm/pmol) was 4 pmol. eIF-2[^{3}H]GDP was α-subunit phosphorylated with [γ-^{32}P]ATP and isolated by Sepharose-heparin chromatography. ● and ●, eIF-2–[^{3}H]GDP; ○ and ○, eIF-2(α-^{32}P)–[^{3}H]GDP. ● and ○, ^{3}H radioactivity; ● and ○, ^{35}S radioactivity. (*D*) Failure of eIF-2(αP) to exchange with SP-bound eIF-2. Samples (200 μl) containing 14 pmol of eIF-2 phosphorylated with ^{32}P in either the α or β subunit and other additions were incubated for 2 min at 30°C and analyzed by sucrose density gradient centrifugation. ○, eIF-2(βP) (control); ●, eIF-2(αP). This experiment was carried out three times with similar results.

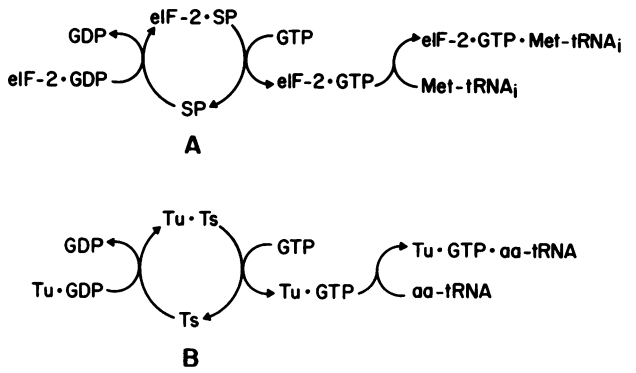

FIG. 6. Schematic representation of the analogy between the initial steps of eukaryotic polypeptide chain initiation (*A*) proposed in this paper and prokaryotic chain elongation (*B*).

eIF-2–SP catalysis of binary complex (Fig. 5 *A* and *B*) as well as GDP-GTP exchange and ternary complex formation (Fig. 5*C*) in the presence of Mg^{2+}. In the absence of Mg^{2+}, these reactions proceed equally well with eIF-2 or eIF-2(αP) with no requirement for eIF-2–SP. This is illustrated in Fig. 5*B* for binary complex formation. eIF-2(αP), which does not form a complex in the presence of Mg^{2+} and eIF-2–SP, does so in their absence, while eIF-2(βP) is active in both cases. Phosphorylation of the α subunit of eIF-2 also blocks the eIF-2 exchange reaction (Fig. 5*D*). These experiments provide support for the view (2–4) that phosphorylation of the α subunit prevents interaction between eIF-2 and the eIF-2-stimulating protein.

DISCUSSION

The first step in eukaryotic polypeptide chain initiation, the formation of the eIF-2–GTP–Met-tRNA$_i$ ternary complex, bears a close resemblance to formation of the ternary complex EF-Tu–GTP–aa-tRNA in prokaryotes (9, 10) or EF-1α–GTP–aa-tRNA in eukaryotes (11). Completion of each round of initiation (elongation) is coupled to GTP hydrolysis and leads to release of the initiation (elongation) factor from the ribosome. In the presence of Mg^{2+}, eIF-2 (18), like EF-Tu (9, 10), has at least a 100-fold greater affinity for GDP than for GTP. EF-Tu is released as EF-Tu–GDP (9, 10) and we have indications that eIF-2 is released as eIF-2–GDP. Our results are consistent with occurrence of the reaction eIF-2–GDP + SP $\rightleftharpoons$ eIF-2–SP + GDP and this reaction is the counterpart of the reaction EF-Tu–GDP + EF-Ts $\rightleftharpoons$ EF-Tu–EF-Ts + GDP in chain elongation (9, 10). We therefore propose that SP, like EF-Ts (or EF-1$\beta\gamma$) acts catalytically (Fig. 6) to permit recycling of eIF-2 in chain initiation, much as EF-Ts (or EF-1$\beta\gamma$) permits recycling of EF-Tu (or EF-1α) in chain elongation. When the α subunit of eIF-2 is phosphorylated, the factor fails to interact with the SP and inhibition of chain initiation ensues. A similar model has been considered before (see, for example, refs. 20 and 21) and has recently been suggested by Clemens *et al.* (22) based on GDP exchange experiments with partially purified eIF-2 ± HCl + ATP.

Thus far we had only small amounts of free SP (8) which, unlike eIF-2–SP, appears to be unstable. Availability of SP in sufficient amounts should make it possible to see whether, like the displacement of EF-Tu- (or EF-1α)-bound GDP by excess EF-Ts (or EF-1$\beta\gamma$) (11, 23), an excess of SP can displace eIF-2-bound GDP.

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1. Weissbach, H. & Ochoa, S. (1976) *Annu. Rev. Biochem.* **45**, 191–216.
2. de Haro, C., Datta, A. & Ochoa, S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 243–247.
3. de Haro, C. & Ochoa, S. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2713–2716.
4. de Haro, C. & Ochoa, S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1741–1745.
5. Ranu, R. S. & London, I. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1079–1083.
6. Das, A., Ralston, R. O., Grace, M., Roy, R., Ghosh-Dastidar, P., Das, H. K., Yaghmai, B., Palmieri, S. & Gupta, N. K. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5076–5079.
7. Ochoa, S., Siekierka, J., Mitsui, K., de Haro, C. & Grosfeld, H. (1981) in *Protein Phosphorylation*, Cold Spring Harbor Conferences on Cell Proliferation, eds. Rosen, O. M. & Krebs, E. G. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 8, pp. 931–940.
8. Siekierka, J., Mitsui, K.-I. & Ochoa, S. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 220–223.
9. Miller, D. L. & Weissbach, H. (1977) in *Molecular Mechanisms of Protein Biosynthesis*, eds. Weissbach, H. & Pestka, S. (Academic, New York), pp. 323–373.
10. Kaziro, Y. (1978) *Biochim. Biophys. Acta* **505**, 95–127.
11. Nagata, S., Motoyoshi, K. & Iwasaki, K. (1978) *J. Biochem.* **83**, 423–429.
12. de Haro, C. & Ochoa, S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2163–2164.
13. Chanduri, A., Stringer, E. A., Valenzuela, D. & Maitra, U. (1981) *J. Biol. Chem.* **256**, 7261–7264.
14. Benne, R., Amesz, H., Hershey, J. W. B. & Voorma, H. O. (1979) *J. Biol. Chem.* **254**, 3201–3205.
15. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
16. Benne, R., Wong, C., Luedi, M. & Hershey, J. W. B. (1976) *J. Biol. Chem.* **251**, 7675–7681.
17. Goldstein, J. & Safer, B. (1979) *Methods Enzymol.* **60**, 165–181.
18. Walton, G. M. & Gill, G. N. (1975) *Biochim. Biophys. Acta* **390**, 231–245.
19. Siekierka, J. (1981) Dissertation (New York Univ., New York).
20. Cherbas, L. & London, I. M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3506–3510.
21. Hunt, T. (1979) in *Miami Winter Symposium: From Gene to Protein*, eds. Russell, T. R., Brew, K., Schultz, J. & Haber, H. (Academic, New York), Vol. 16, pp. 229–243.
22. Clemens, M. J., Pain, V. M., Wong, S. T. & Henshaw, E. C. (1982) *Nature (London)* **296**, 93–95.
23. Miller, D. L. & Weissbach, H. (1970) *Biochem. Biophys. Res. Commun.* **38**, 1016–1022.