Proc. Natl. Acad. Sci. USA
Vol. 75, No. 2, pp. 650–654, February 1978
Biochemistry

Translational control: Recognition of the methylated 5′ end and an internal sequence in eukaryotic mRNA by the initiation factor that binds methionyl-tRNA$_f^{Met}$

(eukaryotic initiation factor 2/cap/globin mRNA/picornavirus RNA/differential recognition of mRNA)

RAYMOND KAEMPFER, HAIM ROSEN, AND RUTH ISRAELI

Laboratory for Molecular Virology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

Communicated by Paul Doty, October 31, 1977

ABSTRACT **Structural analogs of the methylated 5′ end (cap) of eukaryotic mRNA, such as 7-methylguanosine 5′-monophosphate, specifically inhibit both GTP-dependent binding of Met-tRNA$_f^{Met}$ and binding of globin mRNA to eukaryotic initiation factor 2 (eIF-2). Addition of purified eIF-2 effectively relieves the cap analog-induced inhibition of globin mRNA translation. The analog competitively inhibits the function of eIF-2 and of mRNA in protein synthesis. Binding to eIF-2 of capped mRNA as well as noncapped mRNA, such as Mengo virus RNA, can be inhibited completely by free cap molecules, but much more cap is needed to inhibit binding of Mengo virus RNA. mRNA, whether or not it is capped, competitively inhibits the binding of Met-tRNA$_f^{Met}$ to eIF-2.**

These results provide compelling evidence that eIF-2 recognizes mRNA. It is shown that binding of mRNA to eIF-2 is primarily at an internal sequence, and secondarily through the cap. A model for the function of eIF-2 is presented that can account for all these properties. This model can provide a molecular basis for the differential translation of mRNA species, whether or not they are capped.

Most eukaryotic mRNA species of cells and viruses have been shown to possess a unique 5′-terminal structure, 7-methylguanosine(5′)triphosphate(5′)N, termed the cap (1, 2), a notable exception being presented by the mRNAs of picornaviruses (3–6) and satellite tobacco necrosis virus (7). An intact cap structure is thought to be indispensible for the efficient translation of capped mRNA (8–10), although loss of the cap does not always abolish translation (11). Structural analogs of the cap, such as 7-methylguanosine 5′-monophosphate (m^{7}GMP), inhibit translation of capped mRNA species (6, 12, 13) but seem to have little (6) or no (12, 13) effect on translation of noncapped mRNA. The cap is required for stable binding of capped mRNA to ribosomes (6, 12, 14–16), suggesting that it is an important element in the recognition of eukaryotic mRNA.

Elucidation of the mechanism of mRNA recognition is central to the understanding of translational control of eukaryotic gene expression. We have shown that the dependence of initiation of protein synthesis on heme (17), and its sensitivity to inhibition by double-stranded RNA (18), two classical examples of translational control, involve regulation of the activity of eukaryotic initiation factor 2 (eIF-2), the protein that binds methionyl-tRNA$_f^{Met}$ (Met-tRNA$_f$) and GTP during initiation (19). This protein, we have found, possesses unique properties in that it also binds with high affinity to messenger RNA (19, 20). Globin mRNA, R17 phage RNA, Mengo virus RNA, and VSV mRNA all possess a high-affinity binding site for eIF-2, while negative-strand VSV RNA, which does not serve as mRNA, lacks such a site (20). These results indicate that besides binding Met-tRNA$_f$, eIF-2 actively recognizes a specific site in mRNA that is essential for protein synthesis.

Here, we have asked if initiation factor eIF-2 is the protein that recognizes the 5′-terminal cap structure in mRNA. Our finding is that this is indeed the case: free cap structures specifically inhibit the binding of both Met-tRNA$_f$ and mRNA to purified eIF-2, and addition of eIF-2 effectively relieves the cap analog-induced inhibition of translation. We show that cap structures competitively inhibit the function of eIF-2 and of mRNA in protein synthesis. Remarkably, binding of both capped and noncapped mRNA to eIF-2 can be inhibited completely by free cap molecules, but much more cap is needed to inhibit binding of noncapped eukaryotic mRNA. Finally, we show that mRNA, whether or not it is capped, competitively inhibits the binding of Met-tRNA$_f$ to eIF-2.

Our results provide compelling evidence that eIF-2 recognizes not only the 5′-terminal cap structure, but also a second, internal, binding site in mRNA. We present a model for the function of eIF-2 that can account for all of its properties (Fig. 8). In sum, we show that eIF-2 fulfills a crucial, and very likely primary, function in the recognition and binding of mRNA.

RESULTS

Inhibition of Met-tRNA$_f$·GTP·eIF-2 Complex Formation by Cap Analog. Fig. 1 shows that the characteristic function of initiation factor eIF-2, the formation of ternary complexes with Met-tRNA$_f$ and GTP, is inhibited by the cap analog m^{7}GMP but not by m^{7}G or GMP.

Reversal by eIF-2 of Cap Analog-Induced Inhibition of Translation. Protein synthesis in native reticulocyte lysates is relatively resistant to inhibition by cap analogs, presumably because endogenous mRNA already is complexed with initiation factors (16). When endogenous mRNA is first digested by the addition of micrococcal nuclease (21), protein synthesis becomes dependent on the amount of exogenous globin mRNA added (Fig. 2). Translation of this mRNA is readily inhibited by m^7 GMP. The extent of inhibition by m^{7}GMP is greater, the lower the concentration of added mRNA, and the greater the concentration of m^{7}GMP.

Relief of inhibition of translation by m^{7}GMP is observed upon the addition of increasing amounts of eIF-2. Protein synthesis is progressively stimulated in all samples containing m^{7}GMP, but not in the uninhibited controls. Reversal of inhibition is more complete, the greater the concentration of added

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: eIF-2, eukaryotic initiation factor 2; Met-tRNA$_f$, methionyl-tRNA$_f^{Met}$; m^{7}GMP, 7-methylguanosine 5′-monophosphate; m^{7}G, 7-methylguanosine; m^{7}GpppGm, 7-methylguanosine(5′)triphosphate(5′)-2′-*O*-methylguanosine; m^{7}GpppAm, 7-methylguanosine(5′)triphosphate(5′)-2′-*O*-methyladenosine; EMC, encephalomyocarditis; VSV, vesicular stomatitis virus.

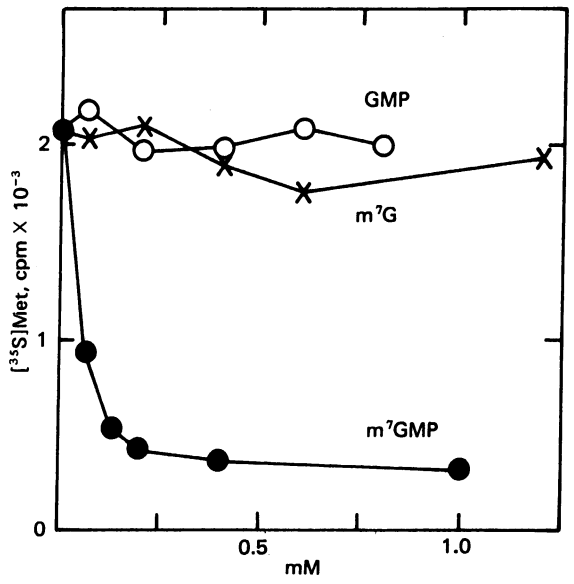

FIG. 1. Effect of cap analog on binding of Met-tRNA$_f$ to eIF-2. GTP-dependent binding of [^{35}S]Met-tRNA$_f$ (3870 cpm) was assayed (20) in a 75-μl mixture containing 3.6 μg of eIF-2 purified as described in the legend for figure 5 of ref. 20, and the indicated concentrations of m^{7}GMP, m^{7}G, or GMP. Background without eIF-2 (30 cpm) was not subtracted.

mRNA, the greater the concentration of eIF-2, and the lower the concentration of m^{7}GMP. This finding demonstrates that inhibition of protein synthesis by the cap analog is competitive with respect to both mRNA and eIF-2.

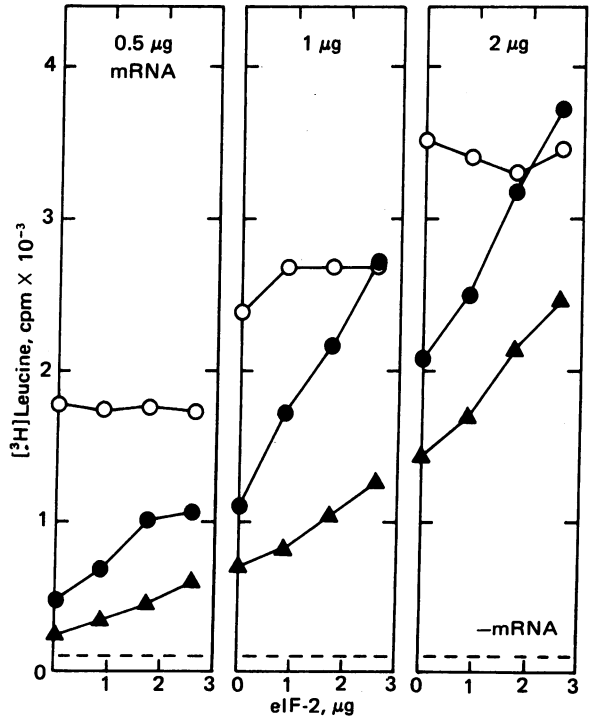

FIG. 2. Relief of m^{7}GMP-induced translational block by eIF-2 and mRNA. A rabbit reticulocyte lysate (17) was incubated with micrococcal nuclease (Sigma) as described (21). Purified globin mRNA (20) was added in the amounts shown. Translation was at 30° in 50-μl reaction mixtures (21) containing the indicated amounts of purified eIF-2 (see Fig. 1) in the absence (○) of m^{7}GMP or in the presence of 1 mM (●) or 2 mM (▲) m^{7}GMP. Hot CCl_3COOH-precipitable [^{3}H]leucine was determined after 60 min. No background (broken line) was subtracted.

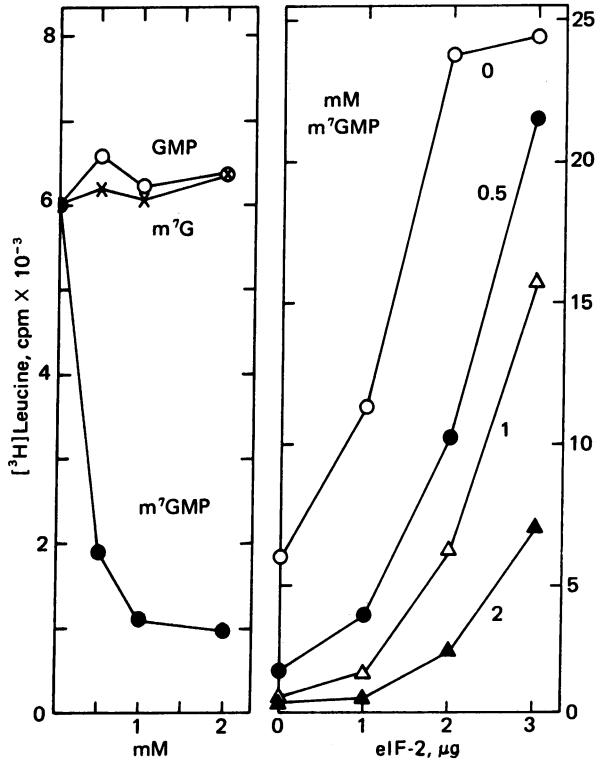

FIG. 3. Effect of m^{7}GMP on eIF-2 dependence of globin mRNA translation. Reticulocyte lysate was centrifuged through a 10-volume bed of Sephadex G-25 in a perforated tube and then incubated with micrococcal nuclease as for Fig. 2. Reaction mixtures (50 μl) contained, besides the indicated amounts of cap analog and purified eIF-2, 30 μl of lysate, 20 μM hemin, creatine kinase at 30 μg/ml, 6.4 mM creatine phosphate, 1.6 mM dithiothreitol, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (pH 7.8), 40 μM spermine, 2 mM Mg(OAc)$_2$, 144 mM KCl, 19 unlabeled amino acids (10 μM each), 2 μCi of [^{3}H]leucine, 0.8 mM ATP, 20 μM GTP, and 1 μg of globin mRNA. Analysis was as in Fig. 2. Background without mRNA was subtracted. (*Left*) Cap analog and related compounds were added as indicated; (*Right*) cap analog was added at the indicated concentrations.

In the experiment of Fig. 3, we have used a reticulocyte lysate that was first subjected to gel filtration and then treated with micrococcal nuclease. As expected, translation of added template in such a lysate is inhibited by m^{7}GMP, but not by m^{7}G or GMP (Fig. 3 *left*). As seen in Fig. 3 *right*, protein synthesis in this filtered lysate is stimulated by the addition of eIF-2, up to a saturation value, even when m^{7}GMP is omitted. In the presence of increasing concentrations of m^{7}GMP, one observes a progressive shift of the eIF-2 dose–response curve to higher values of eIF-2. This experiment provides additional strong evidence that the cap analog inhibits the function of initiation factor eIF-2 in protein synthesis.

Cap Analogs Specifically Inhibit the Binding of Globin mRNA to eIF-2. Globin mRNA, as well as other mRNA species, possesses a high-affinity binding site for eIF-2, as demonstrated by affinity chromatography and equilibrium binding studies (20). We have used ^{125}I-labeled globin mRNA to study the effect of cap analogs on the binding to eIF-2. ^{125}I-Labeled and nonradioactive, native globin mRNA bind with equal affinity to eIF-2 (R. Kaempfer, R. Hollender, H. Soreq and U. Nudel, unpublished data). As seen in Fig. 4 *left*, the formation of complexes between labeled globin mRNA and eIF-2, assayed by nitrocellulose membrane filtration, is inhibited by m^{7}GMP, m^{7}GpppAm, and m^{7}GpppGm, cap structures that inhibit translation, but not by m^{7}G, GMP, or GTP, agents that do not

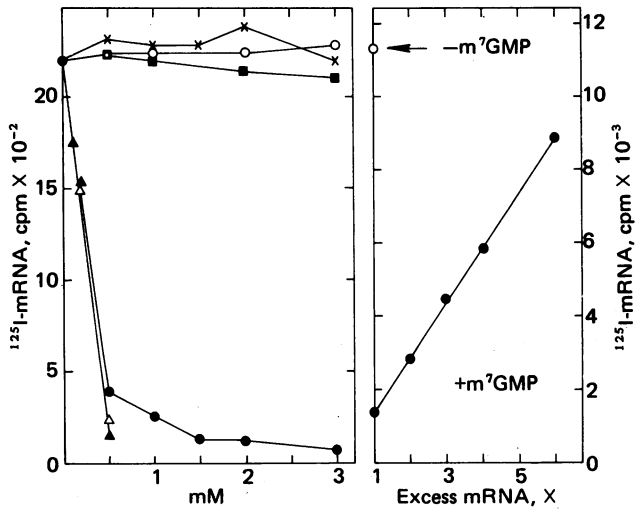

FIG. 4. Effect of cap analogs on binding of globin mRNA to eIF-2. RNA-binding assays (20) contained ^{125}I-labeled purified globin mRNA (20) (0.07 pmol; 4130 cpm), 150 mM KCl, 20 mM Tris·HCl (pH 7.8), 6 mM 2-mercaptoethanol, 0.04 μg of purified eIF-2 (Fig. 1), and cap analogs (all from P-L Biochemicals) at the indicated concentrations. (*Left*) Analogs are: ×, m⁷G; ○, GMP; ■, GTP; ▲, m⁷GpppAm; △, m⁷GpppGm; ●, m⁷GMP. Control without eIF-2 (60 cpm) was subtracted. In experiment on *Right*, 1× globin mRNA is 0.043 pmol, 18,000 cpm; the assays contained 0.04 μg of purified eIF-2 and 2 mM m⁷GMP. Control without eIF-2 (195 cpm) was subtracted.

impair translation. As in the case of translation (Fig. 2), we find that the m⁷GMP-mediated inhibition of globin mRNA binding to eIF-2 can be relieved readily by increasing the concentration of globin mRNA in the binding assay (Fig. 4 *right*). Note that near-complete relief is seen when the mRNA concentration is increased only 6-fold. This experiment demonstrates that mRNA and cap analogs compete for a common binding site on eIF-2.

Cap Analog Inhibits Binding of Noncapped mRNA to eIF-2. It has been reported that translation of noncapped

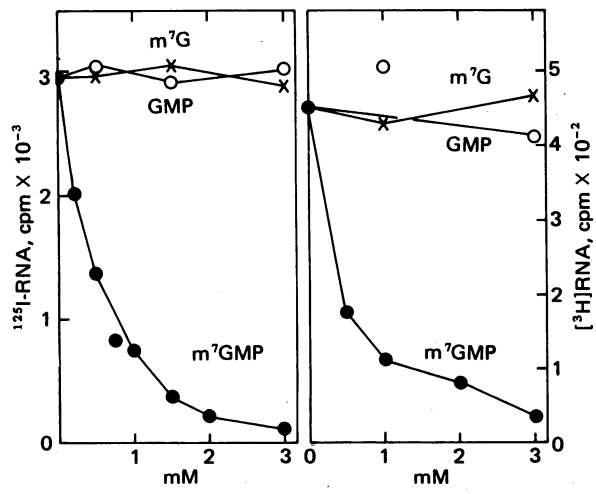

FIG. 5. Effect of cap analog on binding of Mengo virus and Qβ RNA to eIF-2. (*Left*) RNA-binding assays (as in Fig. 4) contained Mengo virus RNA (a gift of F. Brown) labeled with ^{125}I (20) (0.0027 pmol; 3365 cpm), 0.01 μg of purified eIF-2, and cap analogs at the indicated concentrations. Control without eIF-2 (70 cpm) was subtracted. (*Right*) Assays contained Qβ RNA (4 pmol; 2370 cpm) extracted from ^{3}H-labeled virions (a gift of H. Engelberg-Kulka) and 3.9 μg of purified eIF-2. Control without eIF-2 (14 cpm) was subtracted.

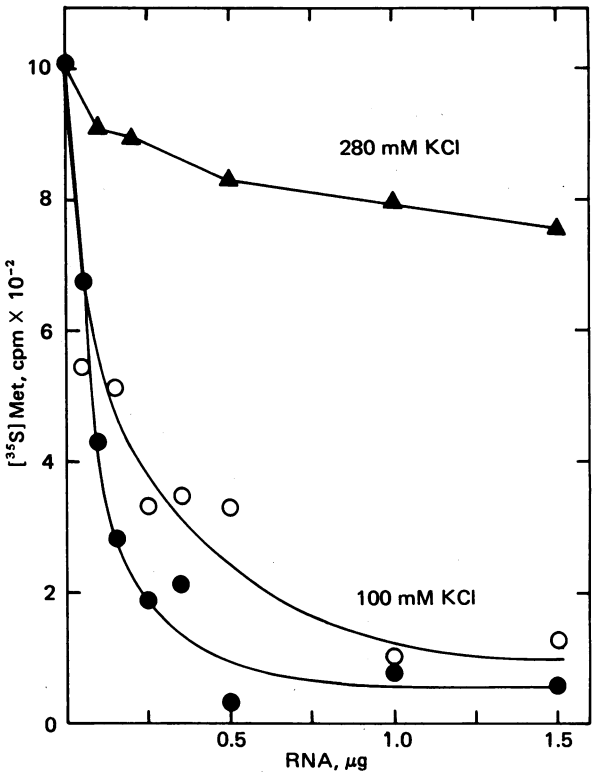

FIG. 6. Effect of R17 phage (○) and globin mRNA (●, ▲) on binding of Met-tRNA$_f$ to eIF-2. The assay was as in Fig. 1, with 3400 cpm of [^{35}S]Met-tRNA$_f$, 2 μg of purified eIF-2, and the indicated amounts of globin mRNA or R17 RNA (20).

mRNA species is not inhibited by cap analogs (6, 12, 13). If that is so, one would expect binding of noncapped mRNA to eIF-2 to be similarly uninhibited. Fig. 5 shows that this is definitely not the case: binding of Mengo virus RNA or Qβ bacteriophage RNA (neither of which is capped) to eIF-2 is completely and specifically inhibited by m⁷GMP. There is, however, one important difference between the inhibition seen here and that observed in Fig. 4 for globin mRNA: to achieve 50% inhibition of complex formation between mRNA and eIF-2, one must add about 50 times more molecules of m⁷GMP per molecule of Mengo virus RNA than in the case of globin mRNA.

mRNA Inhibits Binding of Met-tRNA$_f$ to eIF-2. We have shown that eIF-2 binds both Met-tRNA$_f$ and mRNA with high affinity (20). It was seen in Fig. 1 that an analog of the cap inhibits the binding of Met-tRNA$_f$ to eIF-2. In the experiment of Fig. 6, we show that both globin mRNA and R17 phage RNA are powerful inhibitors of the binding of Met-tRNA$_f$ to eIF-2. Even though R17 RNA is not capped, it is about equally as effective an inhibitor, on a molar basis, as globin mRNA is. This experiment shows that a given eIF-2 molecule cannot stably bind mRNA and Met-tRNA$_f$ at the same time. Note that raising the salt concentration from 100 to 280 mM KCl results in relief of mRNA-mediated inhibition of Met-tRNA$_f$ binding. This is because the half-life of mRNA·eIF-2 complexes decreases drastically with increasing salt concentration (W. R. Abrams and R. Kaempfer, unpublished data), while the binding of Met-tRNA$_f$ to eIF-2 is relatively insensitive.

Cap Analog Slows the Rate of 40S·Met-tRNA$_f$ Complex Formation. It has been reported that cap analogs do not affect the formation of 40S·Met-tRNA$_f$ complexes, but block only the binding of mRNA (14, 16). Yet, in Fig. 1 we show that ternary complex formation, a prerequisite for binding of Met-tRNA$_f$ to 40S ribosomal subunits, is strongly inhibited by m⁷GMP.

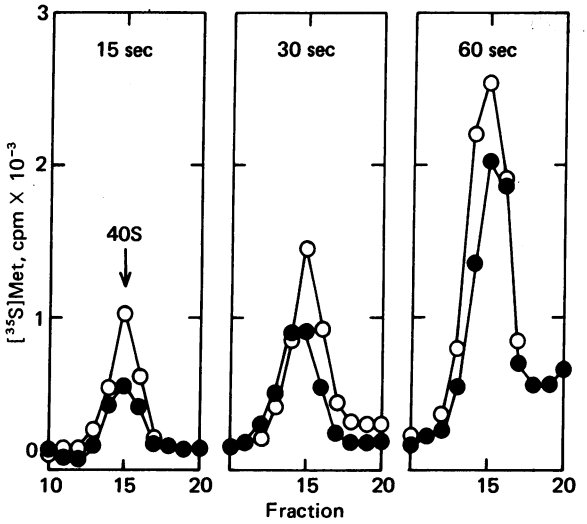

FIG. 7. Effect of m^7GMP on 40S·Met-tRNA$_f$ complex formation. Reticulocyte lysate as used in Fig. 3 was incubated, in the absence of added mRNA, with [^{35}S]methionine (12.5 μCi) with (●) or without (○) 2 mM m^7GMP, in conditions as for Fig. 3. At the indicated times, samples were diluted with 4 volumes of ice-cold 10 mM Tris·HCl (pH 7.4)/1.5 mM $Mg(OAc)_2$/10 mM NaCl and centrifuged for 2.5 hr at 130,000 × *g* through 12.5-ml exponential sucrose gradients (5–13.6%) in the same buffer. Radioactivity precipitated with 5% cold CCl_3COOH was determined for each fraction. Only the 40S region of each gradient (30 fractions) is shown. Sedimentation is from right to left. Profiles are superimposed pairwise.

Because the earlier observations (14, 16) were made after 40S complex formation for 2 and 10 min, respectively, we decided to examine earlier times. Indeed, there is a partial but significant kinetic effect of m^7GMP on 40S·Met-tRNA$_f$ complex formation, most evident after a few seconds, and steadily decreasing by 1–2 min (Fig. 7). Presumably, the additional stabilization of the eIF-2·Met-tRNA$_f$ complex imparted by the 40S subunit and other proteins tends to shift the equilibrium in favor of the 40S complex, even in the presence of m^7GMP. Thus, m^7GMP slows down the rate of 40S·Met-tRNA$_f$ complex formation, but does not block it completely.

DISCUSSION

These results provide compelling evidence that a single protein, initiation factor eIF-2, not only binds Met-tRNA$_f$ during initiation of protein synthesis, but also recognizes with high specificity the 5′-terminal cap structure in eukaryotic mRNA as well as a second, internal site in mRNA. The implication of our findings is that initiation factor eIF-2 fulfills a crucial, and very likely primary function in the essential aspect of translational control, the recognition and binding of mRNA.

The various experimental results in this paper can be understood by consideration of a conceptual model for eIF-2 function, presented in Fig. 8. The eIF-2 molecule, drawn in the center, is seen to possess three binding sites: one for Met-tRNA$_f$, one for the cap, and one for mRNA; we designate the latter as the internal mRNA binding site. All three sites overlap, as evidenced by our demonstration that cap structures inhibit the binding to eIF-2 of Met-tRNA$_f$ as well as mRNA, whether or not it is capped (Figs. 1, 4, and 5), and by our demonstration that mRNA, whether or not it is capped, inhibits the binding of Met-tRNA$_f$ to eIF-2 (Fig. 6). While the cap participates in binding of mRNA to eIF-2, it is clear from our data that the factor does not just bind the cap but also a sequence in mRNA that is different from the cap, and present in noncapped mRNA. The cap binding site, therefore, most likely is not

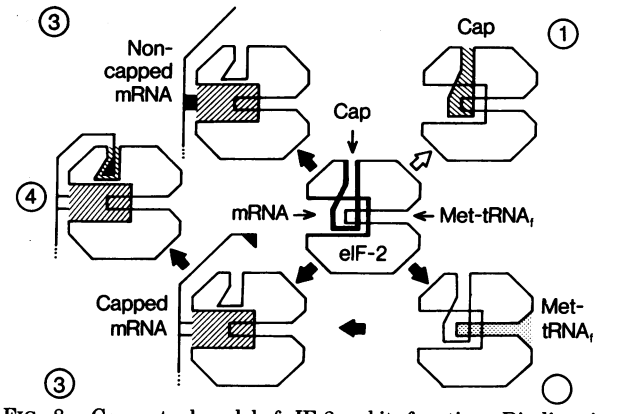

FIG. 8. Conceptual model of eIF-2 and its functions. Binding sites and their overlaps should be interpreted in functional, rather than structural terms. Binding of Met-tRNA$_f$, but not of mRNA, is abolished by *N*-ethylmaleimide, showing a structural difference in binding sites (19, 22).

identical with the internal mRNA binding site of the factor. In the presence of free cap structures, we obtain state *1*, in which the cap site is occupied and binding at the other two sites is prevented. In the presence of Met-tRNA$_f$ and GTP (not shown), we obtain state 2, the classical ternary complex that functions as the first intermediate in initiation of protein synthesis. How does a molecule of capped mRNA bind to eIF-2? If the 5′-terminal cap were to bind first into the cap site, that might result in blocking of the internal mRNA-binding site, because free cap inhibits the binding of noncapped mRNA (Fig. 5). Instead, we propose that binding of mRNA is always first and foremost at the internal mRNA site (state *3 lower*). This binding may induce a conformational change in the eIF-2 molecule such that the cap-binding site and the mRNA-binding site no longer overlap. The 5′-terminal cap of a capped mRNA molecule then enters the cap-binding site, to yield state *4*, and the mRNA molecule is firmly held in position. The presentation of two separate binding states for capped mRNA, 3 and *4*, does not necessarily imply a temporal separation, and indeed the binding most likely occurs in concerted fashion. When binding is to a noncapped mRNA molecule, we propose that precisely the same events occur, to yield state 3 (*upper*), but in this case filling of the cap binding site simply does not occur. It is important to realize that the two states 3 drawn in Fig. 8 are identical, as demanded by the results of Figs. 4 and 5.

During protein synthesis, eIF-2 proceeds from the center through state 2 to states 3 and *4* because binding of Met-tRNA$_f$ is necessary before binding of mRNA can occur (22–24). The results of Fig. 6 imply that when an eIF-2 molecule carrying Met-tRNA$_f$ binds to mRNA, this causes the displacement of Met-tRNA$_f$ from eIF-2. Presumably, this displacement takes place on the 40S ribosomal subunit, where Met-tRNA$_f$ and mRNA are present in equal concentrations.

In inhibiting the function of eIF-2 in protein synthesis, free cap structures behave competitively not only with respect to eIF-2 but also with respect to mRNA (Figs. 2 and 3). The inhibition of mRNA binding to eIF-2 is similarly competitive with mRNA (Fig. 4). It is striking that even though free cap structures are in 10^5-fold molar excess over globin mRNA in Fig. 4, near-total relief of inhibition can be obtained by raising the concentration of globin mRNA only a fewfold. This result must mean that intact globin mRNA has a vastly greater affinity for eIF-2 than even complete cap structures ($m^7GpppGm$ or $m^7GpppAm$). It follows that binding of mRNA to eIF-2 is primarily at the internal mRNA site, and secondarily through the cap, precisely as proposed in our model.

It was noted already in Fig. 5 that, to inhibit binding of a noncapped Mengo virus RNA molecule to eIF-2, one must add about two orders of magnitude more free cap molecules than in the case of globin mRNA. We interpret this to mean that Mengo virus RNA possesses an internal sequence that binds with much higher affinity to the internal mRNA site of eIF-2. Indeed, the absence of a cap in Mengo and other picornavirus RNA species is readily understood in terms of this higher affinity: there is simply no need for the additional stabilization imparted by binding at the cap site (state *4*). Accordingly, we predict that noncapped eukaryotic mRNA species always have a very high affinity for eIF-2 due to their internal structure. This can explain why RNA of encephalomyocarditis virus (EMC), another picornavirus, out-competes cellular mRNA in cell-free translation (25). Our model thus can provide a molecular basis for the extraordinary efficiency of initiation of picornavirus RNA translation in infected cells (26, 27).

If the initiation strength of a mRNA (28) depends on two structural elements, the internal binding sequence and the cap, that would explain why some mRNA species are more dependent on the cap for translation than others, and why removal of the cap causes a smaller decrease in translation of VSV mRNA than of reovirus or globin mRNA (9, 11). Indeed, VSV mRNA is initiated much more efficiently than host mRNA in infected cells (27), even though both mRNA types are capped (2).

We suggest that for a given mRNA the degree of resistance of translation, or of eIF-2 binding, to inhibition by free cap molecules is a good measure of its initiation strength. By this measure, Mengo virus RNA is much stronger than globin mRNA, but Qβ RNA is weaker (see Fig. 5). This is not surprising in view of the prokaryotic nature of Qβ RNA; the related R17 RNA is translated faithfully, but much less efficiently than globin mRNA (29), and indeed it binds an order of magnitude more weakly to eIF-2 (R. Kaempfer, R. Hollender, H. Soreq and U. Nudel, unpublished data). As would be predicted by our model and by that of Lodish (28), m^7GMP differentially inhibits translation of α-globin mRNA over that for β-globin (30).

An important consequence of our findings is that the translation of noncapped eukaryotic mRNA species should be sensitive to inhibition by cap analogs. The reason that this inhibition was missed in several earlier studies (12, 13) is because the ratio of cap analog to mRNA employed was equal to, or at best only 5-fold greater than, that needed to inhibit translation of globin mRNA, yet two orders of magnitude more cap is needed to observe an inhibition of mRNA binding (Fig. 5). Indeed, Canaani *et al.* (6) did obtain partial inhibition of EMC translation by m^7GMP, although they, too, concluded that noncapped mRNA translation was resistant. We calculate, however, from their data that EMC translation was inhibited to the same extent as globin mRNA translation when m^7GMP was in 80-fold greater molar excess; this fits extremely well with the binding data of Figs. 4 and 5. The conclusion of Shafritz *et al.* (16) that binding of EMC RNA to initiation factors is not inhibited by m^7GMP is almost certainly unjustified, because their binding assay contained 60 times more molecules of EMC RNA, and 3 times fewer factor, than in the case of VSV mRNA, and binding of the VSV mRNA was inhibited only by two-thirds. The factor they used was EIF-4B (IF-M3); they failed to observe any inhibition of EIF-2-dependent VSV mRNA binding by m^7GMP (16), presumably because they used about 10^3 times more eIF-2 per mol of VSV mRNA than in our experiments (20). Their suggestion that the cap is recognized by factor eIF-4B does not fit with our data, and possibly is due to contamination with eIF-2. Their factor preparations did not give any reversal of translational inhibition when m^7GMP was in 10^3-fold molar excess over globin mRNA, yet we observe complete reversal by eIF-2 when m^7GMP is in 10^4-fold molar excess over similarly prepared globin mRNA (Fig. 2).

1. Rottman, F., Shatkin, A. & Perry, R. (1974) *Cell* **3**, 197–199.
2. Shatkin, A. (1976) *Cell* **9**, 645–652.
3. Hewlett, M., Rose, J. & Baltimore, D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 327–330.
4. Nomoto, A., Lee, Y. & Wimmer, E. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 375–380.
5. Fellner, P., Frisby, D., Goodchild, A., Porter, A. & Carey, N. (1975) *3rd International Congress of Virology Abstracts*, p. 161.
6. Canaani, D., Revel, M. & Groner, Y. (1976) *FEBS Lett.* **64**, 326–331.
7. Wimmer, E. & Reichmann, M. (1968) *Science* **160**, 1452–1454.
8. Both, G., Banerjee, A. & Shatkin, A. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1189–1193.
9. Muthukrishnan, S., Both, G., Furuichi, Y. & Shatkin, A. (1975) *Nature* **255**, 33–37.
10. Both, G., Furuichi, Y., Muthukrishnan, S. & Shatkin, A. (1975) *Cell* **6**, 185–195.
11. Rose, J. & Lodish, H. (1976) *Nature* **262**, 32–37.
12. Hickey, E., Weber, L. & Baglioni, C. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 19–23.
13. Weber, L., Feman, E., Hickey, E., Williams, M. & Baglioni, C. (1976) *J. Biol. Chem.* **251**, 5657–5662.
14. Roman, R., Brooker, J., Seal, M. & Marcus, A. (1976) *Nature* **260**, 359–360.
15. Both, G., Furuichi, Y., Muthukrishnan, S. & Shatkin, A. (1976) *J. Mol. Biol.* **104**, 637–658.
16. Shafritz, D., Weinstein, J., Safer, B., Merrick, W., Weber, L., Hickey, E. & Baglioni, C. (1976) *Nature* **261**, 291–294.
17. Kaempfer, R. & Kaufman, J. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3317–3321.
18. Kaempfer, R. & Kaufman, J. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1222–1226.
19. Kaempfer, R. (1974) *Biochem. Biophys. Res. Commun.* **61**, 591–597.
20. Kaempfer, R., Hollender, R., Abrams, W. & Israeli, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 209–213.
21. Pelham, H. & Jackson, R. (1976) *Eur. J. Biochem.* **67**, 247–256.
22. Schreier, M. & Staehelin, T. (1973) *Nature New Biol.* **242**, 35–38.
23. Darnbrough, C., Legon, S., Hunt, T. & Jackson, R. (1973) *J. Mol. Biol.* **76**, 379–403.
24. Jay, G. & Kaempfer, R. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3199–3203.
25. Lawrence, C. & Thach, R. (1974) *J. Virol.* **14**, 598–610.
26. Baltimore, D. (1969) in *The Biochemistry of Viruses*, ed., Levy, H. (Marcel Dekker, New York), pp. 103–176.
27. Nuss, D., Oppermann, H. & Koch, G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1258–1262.
28. Lodish, H. (1974) *Nature* **251**, 385–388.
29. Schreier, M., Staehelin, T., Gesteland, R. & Spahr, P. (1973) *J. Mol. Biol.* **75**, 575–578.
30. Suzuki, H. (1976) *FEBS Lett.* **72**, 309–313.