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Role of mRNA competition in regulating translation: Further characterization of mRNA discriminatory initiation factors

(*in vitro* translation/reovirus protein synthesis/cap binding proteins/translation kinetics)

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ABSTRACT **Host and reovirus mRNAs compete with one another for translation in infected cells. Kinetic analysis has suggested that the site of competition is a message discriminatory initiation factor which must bind to the mRNA before it can interact with the 40S ribosomal subunit. The present communication describes an *in vitro* assay which can detect message discriminatory activities. A competitive situation is established by using reovirus and globin mRNAs, and then the specificity with which this competition is relieved by added components is measured. Among the various initiation factors surveyed with this assay, two have the properties expected of the mRNA discriminatory factor. These are eukaryotic initiation factor 4A and a "cap binding protein" complex. Inasmuch as the cap binding protein complex contains a subunit similar or identical to the initiation factor eIF-4A, it seems likely that only one form of the latter factor may be active *in vivo*. *In vitro*, both factors relieve competition among both capped and uncapped reovirus mRNAs according to similar hierarchies. These results suggest that some feature other than the m^7G cap, such as nucleotide sequence or secondary structure, is recognized by the discriminatory factor.**

The fact that different mRNAs may be translated at different rates in eukaryotic cells has been firmly established (1–3). However, the molecular mechanisms that determine translation rates of individual mRNAs are not well understood. To account for mRNA specificity in the initiation step, it has been proposed that mRNAs must compete for a limiting message-discriminatory initiation factor in order to be translated and that competitive inhibition of translation of one mRNA by other mRNAs may be an important factor in regulating their initiation rates (4, 5). It is now clear that mRNA competition plays a central role in the replication of a number of animal viruses, notably encephalomyocarditis virus, vesicular stomatitis virus, and reovirus (4–12). This concept has been applied to uninfected cells as well (13–16).

The precise identification of the message discriminatory factor has remained elusive. Early work using partially purified initiation factor preparations, called IF-M3 and IF-M4, suggested that both were involved in specific mRNA recognition (5, 13). A subsequent change in nomenclature equated IF-M3 with eukaryotic initiation factor 4B (eIF-4B), a M_r 80,000 polypeptide, and IF-M4 with eIF-4A, a M_r 46,000 polypeptide. However, recent studies (ref. 17; unpublished data) showed that IF-M3 contains at least two active components, eIF-4B and one of the "cap binding proteins" [CBP II (18)]. Thus, it was not clear which of these was responsible for the discriminatory activity originally obtained with the IF-M3 preparation.

In the present communication we describe experiments designed to answer this question. Highly purified initiation factors (≥70% pure) were prepared from mouse Krebs ascites tumors, HeLa cells, or rabbit reticulocytes. They were tested for the ability to relieve competition between reovirus mRNAs and globin mRNA *in vitro* according to the specific patterns previously described (19, 20). Our results show that both eIF-4A and CBP II relieve competition specifically and so are candidates for the message discriminatory factor.

MATERIALS AND METHODS

Translation Initiation Factors. Various eukaryotic initiation factors used in the present study have been purified according to published procedures (21–24)—eIF-2, eIF-3, eIF-4A, eIF-4B, and CBP II from rabbit reticulocyte lysate and eIF-2, eIF-3, and eIF-4B from mouse Krebs II ascites and HeLa cell lysates. To assess purity, factors were analyzed by polyacrylamide gel electrophoresis (25) and visualized by the silver stain technique (26).

***In Vitro* Translation System.** Preparation of the fractionated *in vitro* protein-synthesizing system from Krebs ascites cells and all mRNAs has been described (19). The only modification that has been introduced is supplementation with pure factors and reduction in the amount of ribosomal salt wash (RSW) in some of the experiments. Instead of using 20–40 μg of RSW per 25-μl assay we used only 10–15 μg, and the system was saturated (where indicated) with known purified initiation factors. Details of such supplementation are described in *Results*. Ionic conditions were 100 mM potassium chloride and 3 mM magnesium acetate. The protein products synthesized during 2-hr incubations at 30°C were analyzed by polyacrylamide gel electrophoresis and autoradiography as described (19). Various protein bands were then quantitated by excision from the gel, solubili-

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Abbreviations: eIF, eukaryotic initiation factor; CBP, cap binding protein; RSW, ribosomal salt wash; $M_t^{(i)}$, total reovirus mRNA added to a reaction mixture, where "i" denotes a particular type (e.g., S_1, M_3, L_2, etc.); $M^{*(i)}$, reovirus mRNA complexed with discriminatory factor; $M^{(i)}$, free reovirus mRNA; F_t, total discriminatory factor present in a reaction mixture; F, free discriminatory factor; R*, activated 40S ribosomal complex (containing a small ribosomal subunit, Met-tRNA, eIF-2, eIF-3, etc.); $m_t^{(i)}$, $m^{*(i)}$, $m^{(i)}$, f_t, f, and r*, concentrations of the preceding entities defined in uppercase; $K_f^{(i)}$, dissociation constant for $M^{*(i)}$; $Q^{(i)}$, rate of amino acid incorporation into reovirus polypeptide of type i; $Q_0^{(i)}$, rate of amino acid incorporation into reovirus polypeptide of type i in the absence of competing globin mRNA.

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zation with Protosol, and liquid scintillation counting (19).

Specificity of Discriminatory Factor in Competition Relief Experiments as Predicted from Kinetic Theory. The expected effect of adding discriminatory factor to a competitively inhibited system can be calculated from previous data and theory. This can be done either by assuming values for all constants involved (16) or by replotting actual experimental data (20) so as to mimic a competition relief situation. The latter has been done for figure 6C of ref. 20, describing an experiment in which globin mRNA was added in varying amounts to inhibit competitively the translation of capped reovirus mRNA. In this case we assumed that the reduction in translation rate of the reovirus mRNAs was due solely to the sequestration of some of the discriminatory factor by globin mRNA.

If now we consider reversing this process by removing some of the globin mRNA from a competitively inhibited system, the effect will be the same as adding extra discriminatory factor to it. The amount of extra factor made available by reducing the globin mRNA level can be calculated by using equation 23 of ref. 16, which implies the following relationships (terms are defined below or in the Abbreviations footnote):

$$Q_0^{(i)}/Q^{(i)} = m_t^{(i)}/m^{*(i)} \quad [1]$$

$$m_t^{(i)} = m^{*(i)} + m^{(i)} \quad [2]$$

$$\sum_{i=1}^{10} m_t^{(i)} = \sum_{i=1}^{10} m^{*(i)} + \sum_{i=1}^{10} m^{(i)} \quad [3]$$

$$f_t = \sum_{i=1}^{10} m^{*(i)} + f \quad [4]$$

$$K_f^{(i)} = m^{(i)} \cdot f/m^{*(i)} \quad [5]$$

The following quantities are already known: the exact concentrations of each reovirus mRNA, $m_t^{(i)}$; the sum of these concentrations, $\Sigma_{i=1}^{10} m_t^{(i)}$, exactly half-saturates the endogenous discriminatory factor present, f_t; the dissociation constants for complexes of reovirus mRNAs with discriminatory factor $K^{(i)}$; and the translation rates of each individual reovirus mRNA at every level of globin mRNA used, $Q_0^{(i)}$ or $Q^{(i)}$.

Using this information we can proceed as follows. The first statement in the preceding paragraph implies that $\Sigma_{i=1}^{10} m^{*(i)} = f$. Because $K_f^{(i)}$ values all are very low (16, 19), Eq. 5 implies that $\Sigma_{i=1}^{10} m^{(i)}$ is also very low. Therefore, Eq. 3 becomes $\Sigma_{i=1}^{10} m^{*(i)} \simeq \Sigma_{i=1}^{10} m_t^{(i)}$. Using this information we can solve Eq. 4 for f_t. Now, for any value of added globin mRNA, we can use Eq. 1 to calculate $m^{*(i)}$ for any individual reovirus mRNA, because $Q_0^{(i)}$ and $Q^{(i)}$ are available in figure 6C of ref. 20 and $m_t^{(i)}$ is known. These values can now be used in Eq. 2 to calculate $m^{(i)}$. This in turn can be used in Eq. 5 to calculate f. This can be done for every value of added globin mRNA.

The data can now be used to calculate the amount of factor available for translation of reovirus mRNAs as the concentration of competing globin mRNA is reduced. This amount of factor is the sum of f plus $\Sigma_{i=1}^{10} m^{*(i)}$. In order to mimic an experiment in which known amounts of pure factors are added to a competitively inhibited system containing an unknown amount of endogenous discriminatory factor, we have set the value of f plus $\Sigma_{i=1}^{10} m^{*(i)}$ at zero for the highest amount of globin mRNA used (2.0 pmol). Thus normalized, this term can now represent exogenous factor added to the system to relieve competition. As globin mRNA is decreased and competition is relieved, the value of this term increases, and it reaches 0.29 pmol in the absence of globin mRNA. These and intermediate values for this term are arrayed along the abscissa of Fig. 1, where they are called "additional discriminatory factor." The corresponding

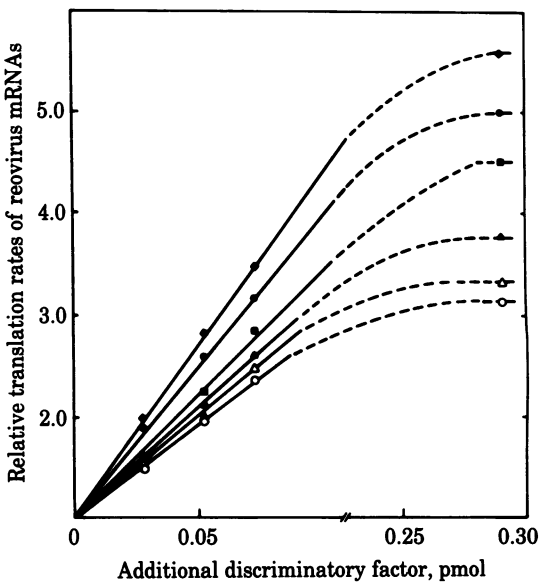

FIG. 1. Hypothetical competition relief assay: the predicted effect on reovirus mRNA translation of addition of excess mRNA discriminatory initiation factor to a competitively inhibited system. A Schreier–Staehelin-type protein synthesis reaction containing a saturating level of globin mRNA (2.0 pmol) and a half-saturating level of reovirus mRNA (0.16 pmol) was analyzed in terms of a previously developed kinetic theory (see *Materials and Methods*). The "relative translation rate" of each reovirus mRNA is defined as the ratio of its translation rates (i.e., the rates of synthesis of its encoded polypeptide) in the presence and absence of additional pure discriminatory factor. This ratio is plotted for each reovirus polypeptide (or mixture of unresolved polypeptides) as a function of added discriminatory factor. The amount of each mRNA present (in pmol), the amount of methionine incorporated into corresponding polypeptides in the absence of additional discriminatory factor (in pmol), and the symbol used for each reoviral polypeptide (or mixture of polypeptides) are: λ_1 plus λ_2 plus λ_3, 0.017, 0.0076, ○; μ_1, 0.024, 0.027, △; μ_{NS}, 0.020, 0.042, ▲; σ_1, 0.010, 0.022, ■; σ_2, 0.015, 0.022, ◆; σ_3 plus σ_{NS}, 0.078, 0.18, ●.

rates of translation of reovirus mRNAs were also normalized by dividing by the rate at maximal competitive inhibition (obtained with 2.0 pmol of globin mRNA). This ratio has been termed the "relative translation rate" and is plotted on the ordinate of Fig. 1. It is evident that the relative translation rates of some mRNAs are influenced much more strongly than those of others by added discriminatory factor, and this differential effect reflects the mRNA specificity of the factor.

This formulation does not presuppose the physical location of the discriminatory factor: equations identical in form to those shown above can be derived from equation 22 of ref. 16, in which the discriminatory site of competition is presumed to be R*. Thus, if the site of competition in our system were in fact R*, then the addition of factors that increase its concentration should produce results identical to those in Fig. 1.

RESULTS

Analysis of CBP II, eIF-4A, and eIF-4B by $NaDodSO_4$/polyacrylamide gel electrophoresis is shown in Fig. 2. The pattern obtained with CBP II indicates that it represents a complex of several polypeptides. One of these is the M_r 24,000 cap binding protein (CBP I, ref. 18). Other major polypeptides in CBP II migrated at M_rs 46,000, 65,000, and 220,000. (The band migrating at M_r approximately 98,000 is a contaminant; that at M_r 36,000 may be a degradation product of one of the larger proteins because its relative amount increases with time.) It is of interest to note that eIF-4A comigrated with the M_r 46,000 pro-

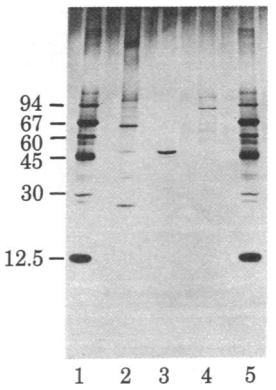

FIG. 2. Electrophoretic analysis of eukaryotic initiation factors. Rabbit CBP II (1 μg) (lane 2), rabbit eIF-4A (0.5 μg) (lane 3), and mouse eIF-4B (0.5 μg) (lane 4) were applied to a 7.5–20% polyacrylamide linear gradient gel. Standard protein markers were applied in lanes 1 and 5; M_r are indicated × 10^{-3}. Minor bands in the M_r 50,000–70,000 range, most apparent in lane 4, are artifacts arising in the electrophoresis procedure.

tein of CBP II, consistent with the suggestion that eIF-4A is one of the polypeptides comprising the CBP II complex (17). Partially purified eIF-4B migrating at M_r 80,000 had several minor contaminants. However, this preparation contained little or no CBP I or II as analyzed by gel electrophoresis or by the highly sensitive crosslinking assay (27) for cap binding proteins. Indeed, none of the constituents in the eIF-4B preparation reacted specifically with oxidized capped mRNA (data not shown).

The effects of discriminatory initiation factors in principle can be measured by several different methods (16). However, their true specificity is most likely to be manifested in a competitive situation: thus, when several mRNAs are in molar excess relative to a factor and must compete with one another for binding to it, the addition of more factor will relieve competition. The translation of each of the various competing mRNAs will be stimulated by added factor according to a hierarchy that is specific for that factor. Such a hierarchy has been carefully studied for the reovirus mRNAs when in competition against globin mRNA (19, 20). This system ideally lends itself to the design of a "competition relief assay" for detecting discriminatory factors. Thus, in the case in which large concentrations of reovirus and globin mRNAs are competing for a limiting amount of a discriminatory factor, the effects of adding factor to the system can be calculated precisely (see *Materials and Methods*).

A typical example of such a calculation is shown in Fig. 1. The salient feature of this figure is that translation of some mRNAs is stimulated much more than that of others by added discriminatory factor. This reflects their different affinities for the factor. Only the addition of the discriminatory factor for which competition occurs will have this particular effect. Factors that nonspecifically increase the rate of any (limiting) steps that follow the competition step (such as the junction of 60S ribosomes) will not have a differential effect. Rather, they will increase the translational rate of each mRNA by the same increment; hence, plotting such data as in Fig. 1 would result in a set of congruent curves (not shown). By contrast, factors that act nonspecifically prior to the competition step may produce a number of different effects, but these will not be similar to the result shown in Fig. 1. Thus, the competition relief assay should respond in a unique way to the addition of the discriminatory factor for which competition occurs, and therefore can be used to identify it. Considerable variation in the assay conditions can be tolerated without jeopardizing its validity. For example, the relative concentrations of reovirus and globin mRNA may be varied, and the latter even may be eliminated, provided that the total mRNA concentration saturates the endogenous discriminatory factor. This follows from the analysis of *in vitro* reaction kinetics in ref. 16.

In the present investigation we used the competition relief assay to detect discriminatory activity in various initiation factors. Typical results obtained from such experiments are shown in Fig. 3. The effects of eIF-4B, eIF-3, and eIF-2 were similar: translation rates of all reovirus mRNAs were stimulated equally, and their dose–response curves were virtually congruent. This indicates that none of these factors acts in a discriminatory fashion, at least under the conditions used here. The maximal stimulation obtained by the addition of excess eIF-2 or eIF-4B was less than 2-fold. This probably indicates that neither factor is severely limiting for translation, even in the presence of satu-

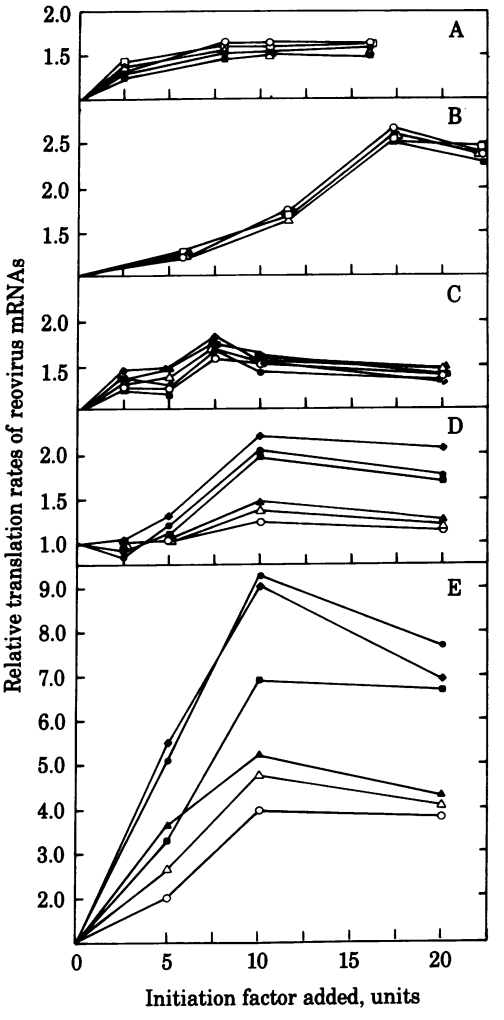

FIG 3. Effects of various eukaryotic initiation factors on the relative translation rates of reovirus mRNAs in a competitively inhibited system. Capped reovirus mRNAs plus globin mRNA were translated in the absence and in the presence of increasing amounts of various eukaryotic initiation factors. The amounts of mRNAs used in each experiment were: *A* and *B*, 0.1 pmol of reovirus and 0.5 pmol of globin: *C*, *D*, and *E*, 0.4 pmol of reovirus and 0.4 pmol of globin. The relative translation rate is equal to the ratio of [^{35}S]methionine incorporated into a given protein in the presence and in the absence of added initiation factor. (*A*) Mouse eIF-4B (1 unit is 0.002 μg); (*B*) mouse eIF-3 (1 unit is 1 μg); (*C*) rabbit eIF-2 (1 unit is 0.01 μg); (*D*) rabbit eIF-4A (1 unit is 0.1 μg); (*E*) rabbit CBP II (1 unit is 0.1 μg). Relative translation rates are indicated by the same symbols as in Fig. 1. Radioactivity incorporated in the absence of added factors was similar to that in Fig. 1.

rating amounts of mRNA. A greater stimulation was seen with added eIF-3. In order to facilitate graphical representation and comparison of the effects of different factors, we have chosen to express the amounts added in terms of arbitrary "units" (see legend to Fig. 3). This procedure enables the comparison of factors of diverse origin, purity, and turnover number in terms of the amount sufficient to saturate the system (this amount was generally 10–20 units).

The negative results obtained with eIF-2, eIF-3, and eIF-4B encouraged us to supplement the basic system with extra amounts of these (5, 20, and 25 units, respectively) in all subsequent assays. This guaranteed that they would not become limiting at some point when increasing amounts of the true discriminatory factor was being tested. When eIF-4A was assayed under these conditions, a differential pattern of stimulation similar to that shown in Fig. 1 was obtained. An even more profound effect was obtained with CBP II. From these results it is clear that both CBP II and eIF-4A have differential effects on mRNA translation. The fact that their effects are so similar to those predicted by competition theory (Fig. 1) strongly suggests that one, or both, of these factors is the discriminatory entity previously defined (5, 12, 13, 16, 19, 20).

These experiments have been repeated in a number of variations. The ratios and absolute concentrations of reovirus and globin mRNAs have been varied widely. Several different preparations of any factor that showed no activity were tested, to ensure the validity of a negative result. Factors were prepared from Krebs ascites tumors, HeLa cells, or rabbit reticulocytes and were produced in three different laboratories (four preparations of eIF-4B, four of eIF-2, three of eIF-3, two of CBP II, and two of eIF-4A were tested).

In order to study whether the effect of eIF-4A and CBP II depends on the cap structure of the mRNAs, we also used uncapped reovirus mRNA in the competition relief assay. In this case, globin mRNA was omitted. Thus, competition occurs only among the uncapped reovirus mRNA species. Stimulatory activity of eIF-4A (Fig. 4*A*) was comparable to that obtained with capped reovirus mRNAs. The effect of CBP II (Fig. 4*B*) also was similar to the pattern obtained with capped mRNAs (the slight differences seen among the σ polypeptides are probably due to experimental error). This result suggests that cap recognition is not necessary for the relief of competition among mRNAs.

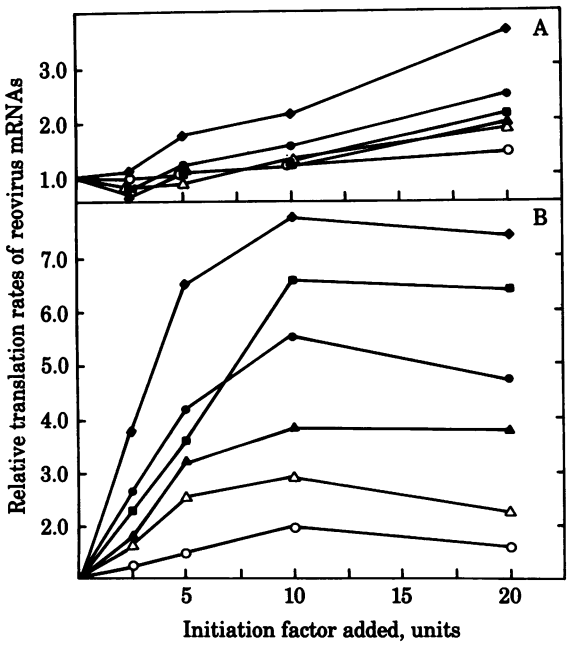

FIG. 4. Effect of eIF-4A (*A*) and CBP II (*B*) on the relative translation rates of uncapped reovirus mRNAs. A saturating amount of uncapped single-stranded reovirus mRNA (0.4 pmol) was translated in the absence and in the presence of increasing amounts of the initiation factors. Protein products were measured and relative translation rates were calculated, as described in Fig. 3. Relative translation rates are indicated by the same symbols as in Fig. 1. Radioactivity incorporated in the absence of added factors was approximately 1/3 the levels indicated in Fig. 1.

Table 1. Effect of various initiation factors in relieving mutual competitive inhibition of globin and reovirus S_3 and M_1 mRNA translation

Initiation factor	Amount of factors added		% inhibition of translation due to presence of competing mRNA		
	μg	Units	Globin	σ_3	μ_1
CBP II	0	0	73	41	32
	0.5	5	56	35	27
	1.0	10	45	27	18
eIF-4A	0	0	73	35	25
	0.5	5	38	25	23
	1.0	10	31	≈0	≈0
eIF-2	0	0	73	41	25
	0.025	2.5	71	48	26
	0.075	7.5	71	38	20
eIF-4B	0	0	75	39	30
	0.01	5	76	37	25
	0.02	10	74	37	27

Saturating amounts of unfractionated single-stranded reovirus mRNA and globin mRNA were translated alone (0.4 pmol) or in equimolar mixtures (0.4 pmol of each). Various rabbit initiation factors were added to the translation systems and the synthesized proteins were measured. Percentage inhibition of translation due to competing mRNA was equal to 1.00 minus the ratio of the amount of a given protein synthesized under competitive conditions (when both types of mRNAs were present) to that synthesized in the absence of competition (when the mRNAs were used alone in the translation system) times 100. In the absence of additional pure factors or reovirus mRNA, 0.14 pmol of methionine was incorporated with 0.4 pmol of globin mRNA. Relative translation rates were influenced by added factors as shown in Fig. 3.

It was desirable to obtain confirmation of these results and conclusions by using different techniques. In particular, it was important to show that the method chosen to plot the data in Figs. 1, 3, and 4 did not bias the result. For this reason we investigated the inhibitory effect of reovirus mRNA on globin synthesis, and the relief of this inhibition by added factors. Under the conditions used here, globin mRNA is a relatively poor initiator (19, 20). When equimolar, saturating amounts of globin and reovirus mRNAs were translated simultaneously, globin synthesis was reduced by 73% (Table 1) by the presence of reovirus mRNA. In contrast, synthesis of reovirus proteins was less affected by the presence of globin mRNA (σ_3 synthesis was reduced by approximately 39% and μ_1 synthesis, by approximately 28%). That this mutual competitive inhibition can be relieved by added CBP II or eIF-4A, but not by eIF-2 or eIF-4B, also is evident in Table 1. Thus, these results confirm the conclusions drawn from Figs. 1, 3, and 4.

In other experiments (data not shown) it has been determined that the differential effect of CBP II is not due to a RNase contaminant because none could be detected. It has also been observed that stimulation of translation by exogenous CBP II only occurs at very high mRNA concentrations. When the mRNA concentration was decreased until it became limiting for trans-

lation (at 0.05 pmol each of globin and reovirus mRNA per assay, for example) addition of CBP II to the system had no effect on the translation of any mRNA. This is the expected result because at subsaturating mRNA concentrations the endogenous CBP II should be sufficient to allow maximal translation rates of all species present (16, 19).

DISCUSSION

The results described above indicate that both eIF-4A and CBP II relieve mRNA competition in a message-specific manner. The specificity for mRNAs exercised by both factors is identical to that previously documented in reovirus translational systems both *in vivo* and *in vitro* (12, 16, 19, 20). These results strongly suggest that both eIF-4A and CBP II are candidates for the discriminatory factor previously defined (5, 13, 16). One possible explanation as to how both factors could play such a role comes from a study of the components of CBP II. Recent results suggest that CBP II is a multiprotein complex and that one of its subunits is similar or identical to eIF-4A (17).

If this is indeed the case, then our results on competition relief become easier to interpret. Several possibilities suggest themselves. (*a*) CBP II may be the active form of the discriminatory factor, in which case eIF-4A added to the *in vitro* system must react with endogenous polypeptides to form more CBP II. (*b*) eIF-4A may be the only discriminatory component in the system and may be able to act either in the free form or as a complex with other proteins (i.e., CBP II). (*c*) CBP II may be inactive as such but may dissociate to produce active eIF-4A, CBP I, etc. This short list by no means exhausts the possibilities, and further experimentation is needed to clarify the situation.

In any event, it is clear that both factors fulfill the prediction, based on *in vivo* (12, 16) and *in vitro* (5) kinetic analysis, that the discriminatory factor binds to mRNA in the absence of 40S ribosomes (ref. 21; unpublished data). (In the case of eIF-4A, both eIF-4B and ATP are necessary cofactors in the mRNA binding reaction.) The implication that 40S ribosomes are not involved in this step is further supported by the observation that addition of eIF-2 or eIF-3, which are components of the R* complex, does not have a discriminatory effect.

Our findings explain earlier results obtained with IF-M3 and IF-M4 (5, 13). The former has been found to contain both eIF-4B and CBP II (refs. 17, 27, 28; unpublished data). The data presented here show that eIF-4B does not have mRNA discriminatory activity, at least in the present system, whereas CBP II does. Thus, it seems likely that the active component of IF-M3 was CBP II. Because IF-M4 was >90% pure, it is clear that its activity was due to the factor now called eIF-4A. We have not been able to obtain CBP I in a pure, stable form, so have not been able to test it for discriminatory activity. [This factor has been shown to specifically stimulate translation of capped mRNAs (28, 29); however, there is no evidence that it recognizes any feature other than the m^7G cap.] No evidence of mRNA specificity was seen with eIF-2 or eIF-3, although these studies were not exhaustive and should be pursued with other types of assays. Message specificity has been reported both for eIF-2 (9, 30) and a component of eIF-3 (31) in different systems. In this connection, it should be noted that Parets-Soler *et al.* (32) have recently described the purification of a factor similar to CBP II that stimulates translation of α-globin mRNA more than that of β-globin mRNA.

It is of interest that the M_r 24,000 and 46,000 subunits of CBP II can be crosslinked to oxidized m^7G caps (ref. 17; unpublished data). Because this reaction is inhibited by the cap analogue, m^7GDP, it suggests that CBP II contains a specific recognition site(s) for the 5′ terminus of capped mRNAs. However, in order to discriminate among capped mRNAs, CBP II must also recognize additional features of the mRNAs with which it interacts. This conclusion is emphasized by the fact that translation of uncapped reovirus mRNAs is stimulated by both CBP II and eIF-4A, with essentially the same specificity as was seen for the capped mRNAs. Thus, it is evident that each mRNA must contain a unique feature or set of features, apart from the cap, which determines its initiation efficiency in a competitive situation. What these features may be is not known, although special nucleotide sequences or structures, or combinations of the two, may be involved.

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