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RNA Unwinding in Translation: Assembly of Helicase Complex Intermediates Comprising Eukaryotic Initiation Factors eIF-4F and eIF-4B

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Ribosome binding to mRNA requires the concerted action of three initiation factors, eIF-4A, eIF-4B, and eIF-4F, and the hydrolysis of ATP in a mechanism that is not well understood. Several lines of evidence support a model by which these factors bind to the 5′ end of mRNA and unwind proximal secondary structure, thus allowing 40S ribosomal subunits to bind. We have previously used an unwinding assay to demonstrate that eIF-4A or eIF-4F in combination with eIF-4B functions as an RNA helicase. To elucidate the molecular mechanism of RNA unwinding, we used a mobility shift electrophoresis assay which allows the simultaneous analysis of unwinding and complex formation between these factors and RNA. eIF-4F forms a stable complex (complex A) with duplex RNA in the absence of ATP. Addition of eIF-4B results in the formation of a second complex (complex B) of slower mobility in the gel. In the presence of ATP, both complexes dissociate, concomitant with the unwinding of the duplex RNA. We present evidence to suggest that unwinding occurs in a processive as opposed to distributive manner. Thus, we conclude that helicase complexes that are formed in the absence of ATP on duplex RNA translocate processively along the RNA in an ATP-dependent reaction and melt secondary structure. These helicase complexes therefore represent intermediates in the unwinding process of mRNA that could precede ribosome binding.

A critical step in eukaryotic protein synthesis is the binding of the small ribosomal subunit to mRNA. This process, considered the overall rate-limiting step in translation (10), requires the participation of at least three initiation factors, eIF-4A, eIF-4B, and eIF-4F, and the hydrolysis of ATP. These factors are believed to bind to the 5′ end of eukaryotic mRNAs and use the energy derived from ATP hydrolysis to denature 5′ proximal mRNA secondary structure, thus facilitating 40S ribosomal subunit attachment (for recent reviews, see references 24 and 26). eIF-4F is a multisubunit complex composed of three polypeptides: (i) eIF-4E a 24-kDa polypeptide which specifically interacts with the cap structure (28); (ii) eIF-4A, a 50-kDa polypeptide characterized as an ATP-dependent RNA helicase (13, 23, 25); and (iii) a p220 polypeptide whose function is not known. Although eIF-4A functions in the free form as a bona fide initiation factor (8), its RNA-dependent ATPase and ATP-dependent RNA helicase activities are enhanced when comprised as a component of the eIF-4F complex. Two forms of eIF-4A mRNA and protein, eIF-4AI and eIF-4AII, have been identified in mouse (19) and rabbit (4) cells. These two forms might possess common and distinct functions, as one of these forms, eIF-4AII, is preferentially localized in the eIF-4F complex (4). eIF-4B is an 80-kDa polypeptide that stimulates the ATPase and RNA helicase activities of both eIF-4A and eIF-4F (1, 2, 7, 23, 25) and has also been implicated in the recycling of eIF-4F components (22). All three factors, eIF-4A, -4B, and -4F, are required for maximal binding of mRNA to 40S ribosomal subunits and for maximal polypeptide synthesis in a fractionated translation system (8).

Recently, Rozen et al. (25) described an RNA unwinding assay to measure the RNA helicase activities of initiation factors. Rabbit reticulocyte eIF-4F or eIF-4A in combination with eIF-4B unwound a synthetic duplex RNA in a bidirectional manner (25). We used this assay to define intermediary steps in the unwinding process by analyzing the assembly of specific complexes that precede RNA duplex unwinding. Here, we show that two distinct complexes are formed between initiation factors and RNA. These complexes are intermediates in the unwinding process of the duplex RNA. We suggest that one of these complexes translocates processively along the duplex RNA and melts its secondary structure.

MATERIALS AND METHODS

RNA unwinding assay. Oligonucleotide templates for in vitro transcription and the RNA helicase assay were as described by Rozen et al. (25), with minor modifications: RNA transcription mixtures contained 320 nM DNA template and 1 mM each ATP, CTP, and UTP, 200 μM GTP and [α-^{32}P]GTP (200 μCi; 3,000 Ci/mmol), and 200 U of T7 RNA polymerase (New England Biolabs, Inc.) in a final volume of 100 μl. The substrate used for RNA unwinding consisted of an uncapped duplex RNA containing a 10-bp double-stranded region at its 5′ end and single-stranded regions of 30 nucleotides at its 3′ end. For the helicase assay, initiation factors eIF-4A, eIF-4B, and eIF-4F, purified from rabbit reticulocytes (8), were incubated with 100 to 200 pg (1,000 to 2,000 cpm) of ^{32}P-labeled uncapped RNA for 10 min at 37°C in buffer containing 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-KOH (pH 7.5), 70 mM KCl, 2 mM dithiothreitol, 1 mM ATP, 0.5 mM magnesium acetate, 0.1 mM GTP, 5% glycerol, and 20 U of RNAsin (Promega Biotec) in a final volume of 10 μl. The reaction was termi-

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nated by the addition of 2.5 μl of a solution containing 50% glycerol, 2% sodium dodecyl sulfate (SDS), 20 mM EDTA, and xylene cyanol-bromophenol blue dye. The reaction mixture was applied to a native 0.75-mm-thick 8% polyacrylamide gel (50:1, acrylamide/bisacrylamide), containing 5% glycerol in 0.5× Tris-borate-EDTA (TBE), which had been preelectrophoresed for 30 min at 30 mA. Electrophoresis was carried out in 0.5× TBE at constant current of 25 mA for 2.5 h at 4°C. Dried gels were exposed against Fuji RX film at −70°C for 24 to 48 h. Unwinding efficiency is defined as the ratio of unwound monomer RNA relative to duplex RNA. Quantitation was performed by scanning autoradiograms in the linear range of exposure of the X-ray film with a soft laser scanning densitometer (LKB Instruments Inc.).

Mobility shift electrophoresis assay. The duplex RNA unwinding assay was performed, and the reaction was stopped by the addition 2.5 μl of a solution containing 50% glycerol, 20 mM EDTA, and 0.25 mg of tRNA per ml and analyzed by electrophoresis as described above. RNA-protein complexes were quantitated by soft laser scanning densitometry (LKB Instruments Inc.).

RESULTS

Translation helicase complexes. To study specific complexes which might serve as prerequisite intermediates in the unwinding of mRNA 5′-end-proximal secondary structures, we used a previously described RNA helicase assay (25). Uncapped RNA molecules were produced by synthetic oligonucleotide-directed T7 RNA transcription which form a duplex RNA composed of 10 alternating G and C residues (free energy of formation, approximately −30 kcal [ca. −126 kJ]) at their 5′ ends and a single-stranded extension of 30 nucleotides at their 3′ ends. To test for factor-mediated unwinding, the radiolabeled RNA duplex was incubated with purified initiation factors and analyzed by native polyacrylamide gel electrophoresis for duplex dissociation. To measure the extent of conversion of duplex RNA to the monomer form, the reaction was stopped by the addition of SDS and EDTA to disrupt protein-RNA complexes (25). To examine protein-RNA complexes, the addition of SDS before gel analysis was omitted and protein-RNA complexes were discerned by the retarded mobility of the RNA. This procedure is schematically shown in Fig. 1.

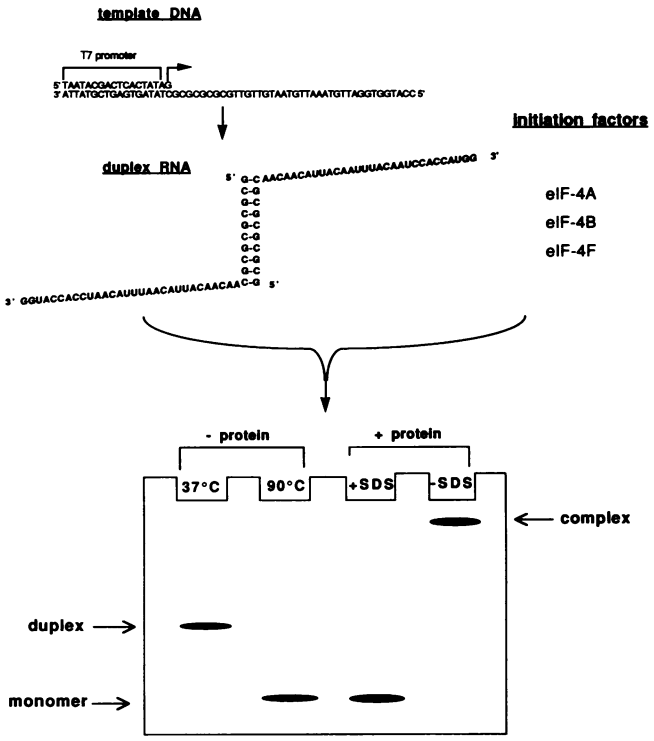

FIG. 1. Scheme of the duplex RNA unwinding/mobility shift gel electrophoresis assay.

Complex formation was tested with different combinations of initiation factors that are active in the unwinding assay (Fig. 2). Migrations of the duplex and monomer forms of the RNA are shown in lanes 1 and 2, respectively. At 37°C, approximately 95% of the input RNA exists in the duplex form (lane 1). It was previously shown that the combination of rabbit reticulocyte eIF-4F and -4B or eIF-4A and -4B was able to unwind duplex RNA in an ATP-dependent manner (25). To analyze the assembly of specific complexes that precede RNA duplex unwinding, combinations of initiation factors were first incubated with duplex RNA in the absence of exogenously added ATP. Incubation of the duplex RNA with eIF-4B by itself (lane 4) or in combination with eIF-4A (lane 3) did not yield any detectable complexes under the conditions used in this assay. However, as previously reported (25), eIF-4A in combination with eIF-4B was able to effect the unwinding of the duplex RNA in the presence of ATP (data not shown). It is likely that complexes between a combination of eIF-4A plus eIF-4B and RNA are formed prior to unwinding but are presumably unstable under our gel electrophoresis conditions. RNA-protein complexes could be readily detected, however, when eIF-4B and eIF-4F were incubated together with duplex RNA (lane 5). Incubation of eIF-4F alone with the duplex RNA in the absence of ATP resulted in the formation of a complex, complex A (lane 6). Incubation of eIF-4B and eIF-4F with duplex RNA, in the absence of ATP, yielded a second, slower-migrating complex, complex B (lane 5). As previously reported (25), the combination of eIF-4F and e[illegible]-4B, but not eIF-4F alone, was sufficient to effect duplex RNA unwinding in the presence of ATP (data not shown). It is possible, although unlikely, that the composition of these complexes is due to trace protein contaminations in the initiation factor preparations. The use of recombinant factors, when available, would be necessary to definitely rule out this possibility.

We wanted to rule out any possible involvement of ATP hydrolysis in complex formation. ATP might be present in trace amounts in the initiation factor preparations. Therefore, the gel electrophoresis shift assay was repeated in the presence of the nonhydrolyzable analog of ATP, AMP-PCP (Fig. 3). eIF-4F formed a stable complex (complex A) with duplex RNA (lane 3) under these conditions. Addition of eIF-4B after prior incubation with eIF-4F resulted in the immediate formation of complex B (lane 4). Complexes A and B are relatively stable (lane 5; generally, the complexes are even more stable than are shown in this figure). Of significance is the finding that analysis of the RNA in the complex, following the addition of SDS, revealed that the duplex RNA remained completely intact (lane 6). This result clearly shows that although ATP hydrolysis is required for unwinding of the duplex RNA, it is not required for complex formation. The lack of requirement for ATP hydrolysis is also consistent with the fact that complexes A and B are capable of forming at 0°C (data not shown), indicative of a

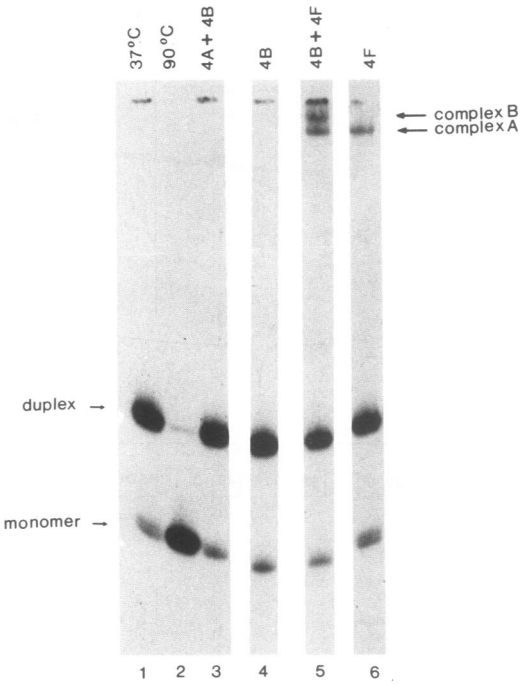

FIG. 2. Formation of helicase complexes with initiation factors in the absence of ATP. The mobility shift gel electrophoresis assay was performed following incubation for 10 min at 37°C with combinations of rabbit reticulocyte eIF-4A (3 μg), eIF-4B (0.5 μg), and eIF-4F (1 μg) and 100 pg of radiolabeled duplex RNA as indicated. Lane 1, RNA incubated for 10 min at 37°C; lane 2, RNA incubated for 5 min at 90°C.

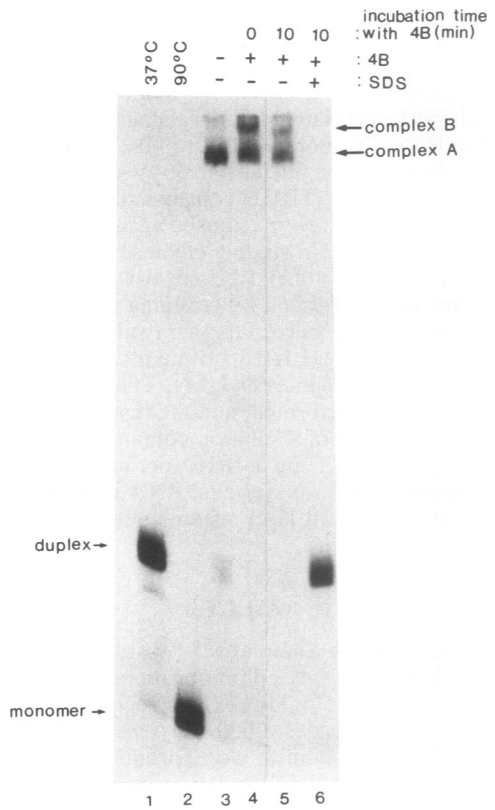

FIG. 3. RNA-protein complex formation in the presence of AMP-PCP. eIF-4F (1 μg) was incubated with 100 pg of duplex RNA for 10 min (lane 3). eIF-4B (0.5 μg) was then added where indicated, and aliquots were removed after the indicated times of incubation and analyzed by gel electrophoresis. AMP-PCP was present at a final concentration of 1 mM. SDS was added prior to electrophoresis where indicated. Lane 1, RNA incubated for 10 min at 37°C; lane 2, RNA incubated for 5 min at 90°C.

reaction with a low energy of formation. Also, the immediate formation of these complexes suggests that the rate-limiting step in duplex RNA unwinding involves a step subsequent to complex formation.

Translation unwinding complexes dissociate in the presence of ATP. Next, we determined the effect of ATP on complex formation vis-à-vis the unwinding activity. A time course of the fate of the complexes formed between the combination of eIF-4F plus eIF-4B and RNA, in the presence of ATP, is shown in Fig. 4. Complex A formed immediately upon the addition of eIF-4F (lane 3) and was stable after 10 min of incubation in the presence of ATP (lane 4). (A minor slower-migrating complex is also seen, especially at 0 min. The appearance of this band was not reproducible in other experiments.) eIF-4B was then added, and aliquots were removed after various time periods. Complex B formed immediately after addition of eIF-4B (lane 5). After 2 min of incubation with eIF-4B, the majority (~70%) of complexes A and B remained intact (lane 6), and analysis of duplex RNA unwinding after the addition of SDS revealed only partial unwinding (~20%; lane 7). However, after 10 min of incubation with eIF-4B, most of complexes A and B dissociated (>95%), concomitant with the dissociation of the duplex RNA (lane 8). Thus, the RNA-protein complexes are very likely intermediates in the unwinding process, inasmuch as their disappearance correlates temporally with the unwinding of the duplex RNA. These results also suggest that the formation of a stable complex A (containing eIF-4F) precedes that of complex B. When the order of addition of initiation factors was reversed, i.e., when RNA was first preincubated with eIF-4B, complexes were not formed (Fig. 2). However, when eIF-4F was added, complexes A and B formed immediately (data not shown).

Stability of eIF-4F–duplex RNA complexes. The previous results suggest that ATP hydrolysis is required for unwinding but not for the binding of initiation factors to the duplex RNA. However, ATP might affect the turnover of the complex without affecting net stability. To address this question, we compared the effect of ATP on the stability of the complexes as measured by competition with excess ^{3}H-labeled RNA over a 30-min time period (Fig. 5). Complex A was formed, followed by the addition of excess ^{3}H-labeled RNA and further incubation prior to gel analysis. Approximately half of the labeled complex remained after the addition of a 10-fold excess of unlabeled RNA duplex in the presence or absence of ATP (lanes 5 and 9), and about 80% of the complex was competed out with a 50-fold molar excess of RNA in the presence (lane 10) or absence (lane 6) of ATP. The requirement for a 50-fold excess of ^{3}H-labeled RNA to effectively displace the ^{32}P-labeled duplex from eIF-4F suggests active displacement of the prebound ^{32}P-labeled RNA by competitor RNA. The similar degrees of displacement of the ^{32}P-labeled complex with a 50-fold excess of ^{3}H-labeled RNA in the presence and absence of ATP suggest that the off-rate of complex A is not affected by ATP.

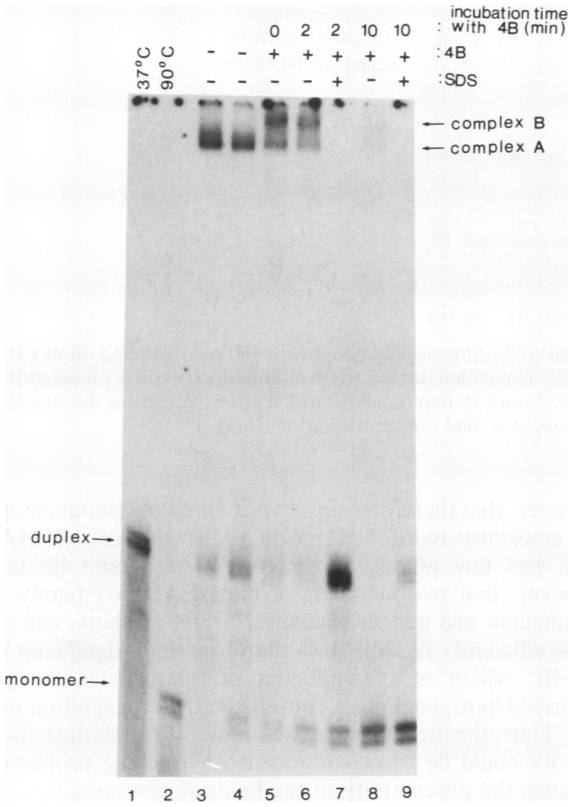

FIG. 4. Kinetics of RNA-protein complex formation and duplex RNA unwinding in the presence of ATP. eIF-4F (1 μg) was incubated with 100 pg of duplex RNA for 0 min (lane 3) or 10 min (lane 4) in the presence of 1 mM ATP. eIF-4B (0.5 μg) was then added where indicated, and aliquots were removed at different time intervals as indicated. SDS was added prior to gel electrophoresis where indicated. Lane 1, RNA incubated for 10 min at 37°C; lane 2, RNA incubated for 5 min at 90°C.

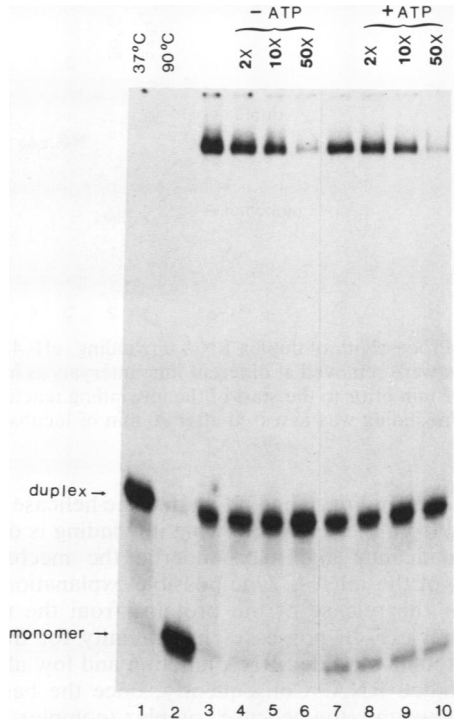

FIG. 5. Turnover of the eIF-4F–RNA complex in the presence or absence of ATP. eIF-4F (1 μg) was preincubated with 100 pg of ^{32}P-labeled duplex RNA for 10 min in the absence (lanes 3 to 6) or presence (lanes 7 to 10) of 1 mM ATP. Various excess amounts of [^{3}H]RNA (as indicated) or buffer (lanes 3 and 7) were added, incubation continued for a further 30 min, and the sample was analyzed for complex formation.

Initiation factor-mediated duplex RNA unwinding is processive. DNA helicases function through both processive and nonprocessive mechanisms (for a recent review, see reference 16). To determine whether initiation factor-mediated RNA helicase activity is processive, we examined the effect of excess unlabeled duplex RNA on the unwinding of the ^{32}P-labeled duplex RNA when added at various time periods during incubation. It is anticipated that if unwinding is processive, addition of excess unlabeled substrate after complex formation has occurred should not affect unwinding. Figure 6 shows the time course of duplex RNA unwinding by the combination of eIF-4F and eIF-4B over a 20-min time period (lanes 3 to 8). This reaction was significantly inhibited (~4-fold) in the presence of excess (~100-fold) unlabeled RNA when this RNA was added prior (2 min) to the addition of radiolabeled substrate (lane 9). However, the addition of excess unlabeled RNA failed to effectively inhibit (only ~1.5-fold inhibition) duplex RNA unwinding when added 1, 2, or 5 min after the onset of the unwinding reaction (lanes 10 to 12). This result suggests that the combination of eIF-4F and eIF-4B acts in a processive manner to unwind duplex RNA, similar to that observed for the DNA helicase activity of large T antigen of simian virus 40 and other DNA helicases (29). A similar result was also obtained for the action of eIF-4A- and eIF-4B-mediated duplex RNA unwinding (data not shown).

DISCUSSION

Using a gel shift electrophoresis assay in combination with an RNA unwinding assay (25), we demonstrate the assembly of specific RNA-protein complexes involved in the unwinding of double-stranded RNA. The formation of these complexes is likely to be an intermediate step in RNA secondary structure unwinding. eIF-4F is capable of forming a stable complex (complex A) in the absence of ATP. This complex is stable in the presence of ATP, consistent with the inability of eIF-4F to independently unwind duplex RNA (25). Addition of eIF-4B results in the formation of an additional, slower-migrating complex (complex B). We assume that complex B contains both eIF-4F and eIF-4B proteins. Both complexes dissociate upon incubation with ATP, concomitant with the unwinding of the duplex RNA. However, the kinetics of complex A disappearance appears to be somewhat faster than that of complex B (Fig. 4). One possible explanation is that the ATP facilitates the conversion of complex A to complex B. We propose that complex B, which is most probably the active helicase, then tracks along the duplex RNA in a processive manner, thereby dissociating the base-paired nucleotides, and is released from the RNA upon completion of unwinding.

We have observed similar complexes with duplex RNAs that contain 5′ single-stranded ends (unpublished results). Consequently, these results could serve as a model system

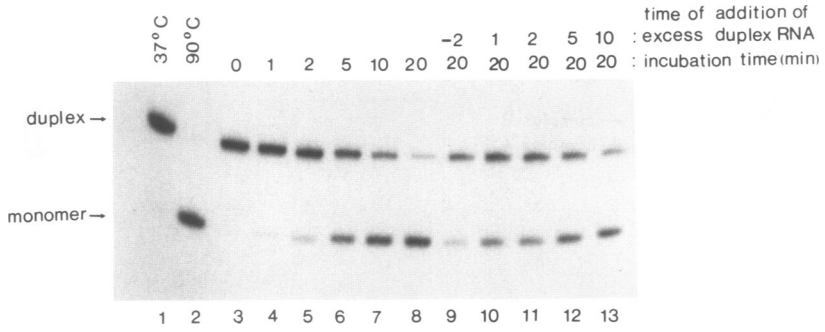

FIG. 6. Processivity of duplex RNA unwinding. eIF-4F (0.5 μg) and eIF-4B (0.2 μg) were incubated with 100 pg of labeled duplex RNA, and aliquots were removed at different time intervals as indicated (lanes 3 to 8). Unlabeled duplex RNA (100-fold excess over labeled RNA) was added 2 min prior to the start of the unwinding reaction (lane 9) or at 1, 2, 5, and 10 min (lanes 10 to 13) after the start of the unwinding reaction. Unwinding was assessed after 20 min of incubation. ATP was present at a final concentration of 1 mM.

for mRNA unwinding. The finding that the helicase complex is released from the RNA following unwinding is of considerable significance and may underlie the mechanism of unwinding of the mRNA. One possible explanation, among others, for the release of the proteins from the unwound RNA is that eIF-4F possesses high affinity for the single-stranded/double-stranded RNA junction and low affinity for single-stranded RNA. Consequently, once the base-paired region is unwound, the helicase complex (complex B) dissociates from the RNA. This interpretation is consistent with our recent results showing that eIF-4F binds more readily to the duplex RNA than to the single-stranded RNA generated from the duplex RNA following heat denaturation (11b). Another, less likely explanation for the helicase dissociation is that the helicase complex migrates along the RNA until it reaches the end and then falls off. The first possibility is more consistent with the unwinding of mRNA, where the translocating helicase complex is unlikely to move along the entire length of the mRNA. A reasonable hypothesis to explain the mode of helicase action on the mRNA posits that the helicase complex unwinds a limited stretch of duplex RNA. Assisted by initiation factors, the resultant single-stranded RNA functions as a ribosome landing pad. Following binding, the 40S ribosome may migrate on the mRNA (if not bound directly to the initiator AUG), aided by the action of eIF-4A and eIF-4B until it encounters the appropriate initiator AUG. This important issue needs to be clarified by further experimentation.

eIF-4B complements both eIF-4A and eIF-4F in the RNA helicase assay (13, 23, 25). However, only incubation of the combination of eIF-4F and -4B with RNA generates stable RNA-protein complexes that could be readily detected by the gel shift assay. It is therefore likely that other components of the eIF-4F complex (e.g., p220) are important for the formation of stable complexes. The greater stability of complexes containing eIF-4F is consistent with the finding that unwinding by the combination of eIF-4F and eIF-4B is more efficient than the unwinding by eIF-4A and eIF-4B (25).

The helicase function described here combines the activities of two proteins that interact with RNA to effect a biochemical activity. These proteins have properties that are shared by two large families of proteins; eIF-4A belongs to a family containing a growing number of proteins (~30 members), termed the DEAD box family, whose members, on the basis of their homology to the founder member, eIF-4A, are believed to possess helicase activity (14). It is of interest, however, that these proteins do not contain a ribonucleoprotein consensus motif (3, 12). Consistent with this, eIF-4A by itself has low affinity for RNA (7) (it should be noted however, that two members of the DEAD box family, the mammalian p68 and the bacterial SrmB proteins, can bind RNA efficiently [9, 20]). It is therefore very significant that eIF-4B, which is a component of the helicase complex described here, contains a consensus RNA recognition motif (18). Thus, the mechanism of action of eIF-4B in the helicase activity could be to provide an RNA tracking property or enhance the processivity of the helicase complex.

On the basis of the results presented here and in previous reports (for reviews, see references 24 and 26), a model is presented to explain the role that these initiation factors play in 40S ribosomal subunit binding to mRNA. The first step involves eIF-4F binding to the mRNA through the interaction of eIF-4E with the cap structure. This is followed by eIF-4B binding. In an ATP-driven reaction, these factors unwind the secondary structure in the 5′ untranslated region of the mRNA. Notwithstanding its low affinity for RNA, eIF-4A is postulated to coat the newly created single-stranded regions of the mRNA (1, 8, 26), chiefly because it is present in excess to other components of the translational machinery (5). This may be necessary to maintain the unstructured state of the mRNA to facilitate 40S ribosomal subunit binding.

A similar mechanism is likely to function during internal binding of ribosomes to mRNA (27). In this process, eIF-4A could substitute for eIF-4F, and the initial interaction of the helicase complex is with an internal sequence in the 5′ untranslated region of the mRNA (21). The interaction of eIF-4A and eIF-4B with RNA is weaker than that of the combination of eIF-4F and eIF-4B, as evidenced from our inability to detect stable complexes containing eIF-4A and eIF-4B by the gel electrophoresis mobility shift assay (Fig. 2). It is possible, however, that RNA binding and helicase activities of eIF-4A and eIF-4B are enhanced by auxiliary proteins, such as p52 and p57, two proteins that bind to sequences in the ribosome landing pads of poliovirus, encephalomyocarditis virus, and foot-and-mouth disease virus mRNAs (11, 15, 17).

The identification of RNA-protein complexes as early intermediates in translation initiation should provide insight into this intricate process and the numerous regulatory pathways that modulate translation rates.

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REFERENCES

1. **Abramson, R. D., T. E. Dever, T. G. Lawson, B. K. Ray, R. E. Thach, and W. C. Merrick.** 1987. The ATP-dependent interaction of eukaryotic initiation factors with mRNA. J. Biol. Chem. **262**:3826–3832.
2. **Abramson, R. D., T. E. Dever, and W. C. Merrick.** 1988. Biochemical evidence supporting a mechanism for cap-independent and internal initiation of eukaryotic mRNA. J. Biol. Chem. **263**:6016–6019.
3. **Bandziulis, R. J., M. S. Swanson, and G. Dreyfuss.** 1989. RNA-binding proteins as developmental regulators. Genes Dev. **3**:431–437.
4. **Conroy, S. C., T. E. Dever, C. L. Owens, and W. C. Merrick.** 1990. Characterization of the 46,000 dalton subunit of eIF-4F. Arch. Biochem. Biophys. **282**:363–371.
5. **Duncan, R., S. C. Milburn, and J. W. B. Hershey.** 1987. Regulated phosphorylation and low abundance of HeLa cell initiation factor eIF-4F suggest a role in translational control. J. Biol. Chem. **262**:380–388.
6. **Edery, I., M. Hümbelin, A. Darveau, K. A. W. Lee, S. Milburn, J. W. B. Hershey, H. Trachsel, and N. Sonenberg.** 1983. Involvement of eIF-4A in the cap recognition process. J. Biol. Chem. **258**:11398–11403.
7. **Grifo, J. A., R. D. Abramson, C. A. Satler, and W. C. Merrick.** 1984. RNA-stimulated ATPase activity of eukaryotic initiation factors. J. Biol. Chem. **259**:8648–8654.
8. **Grifo, J. A., S. M. Tahara, M. A. Morgan, A. J. Shatkin, and W. C. Merrick.** 1983. New initiation factor activity required for globin mRNA translation. J. Biol. Chem. **258**:5804–5810.
9. **Hirling, H., M. Schneffner, T. Restle, and H. Stahl.** 1989. RNA helicase activity associated with human p68 protein. Nature (London) **339**:562–564.
10. **Jagus, R., W. F. Anderson, and B. Safer.** 1981. The regulation of initiation of mammalian protein synthesis. Prog. Nucleic Acid Res. Mol. Biol. **25**:127–185.
11. **Jang, S. K., and E. Wimmer.** 1990. Cap-independent translation of encephalomyocarditis virus RNA: structural elements of the internal ribosomal entry site and involvement of a cellular 57-kD RNA-binding protein. Genes Dev. **4**:1560–1572.

11a. **Jaramillo, M.** Unpublished data.

12. **Kenan, D. J., C. C. Query, and J. D. Keene.** 1991. RNA recognition: towards identifying determinants of specificity. Trends Biochem. Sci. **16**:214–220.
13. **Lawson, T. G., K. A. Lee, M. M. Maimone, R. D. Abramson, T. E. Dever, W. C. Merrick, and R. E. Thach.** 1989. Dissociation of double-stranded polynucleotide helical structures by eukaryotic initiation factors, as revealed by a novel assay. Biochemistry **28**:4729–4734.
14. **Linder, P., P. F. Lasko, P. Leroy, P. J. Nielsen, K. Nishi, J. Schnier, and P. P. Slonimski.** 1989. Birth of the D-E-A-D box. Nature (London) **337**:121–122.
15. **Luz, N., and E. Beck.** 1990. A cellular 57 kDa protein binds to two regions of the internal translation initiation site of foot-and-mouth disease virus. FEBS Lett. **269**:311–314.
16. **Matson, S. W., and K. A. Kaiser-Rogers.** 1990. DNA helicases. Annu. Rev. Biochem. **59**:289–329.
17. **Meerovitch, K., J. Pelletier, and N. Sonenberg.** 1989. A cellular protein that binds to the 5′-noncoding region of poliovirus RNA: implications for internal translation initiation. Genes Dev. **3**:1026–1034.
18. **Milburn, S. C., J. W. B. Hershey, M. V. Davies, K. Kelleher, and R. Kaufman.** 1990. Cloning and expression of eukaryotic initiation factor 4B cDNA: sequence determination identifies a common RNA recognition motif. EMBO J. **9**:2783–2790.
19. **Nielsen, P. J., and H. Trachsel.** 1988. The mouse protein synthesis initiation factor 4A gene family includes two related functional genes which are differentially expressed. EMBO J. **7**:2097–2105.
20. **Nishi, K., F. Morel-Deville, J. W. B. Hershey, T. Leighton, and J. Schnier.** 1988. An eIF-4A-like protein is a suppressor of an *Escherichia coli* mutant defective in 50S ribosomal subunit assembly. Nature (London) **336**:496–498.
21. **Pelletier, J., and N. Soneneberg.** 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature (London) **334**:320–325.
22. **Ray, B. K., T. G. Lawson, R. D. Abramson, W. C. Merrick, and R. E. Thach.** 1986. Recycling of messenger RNA cap-binding proteins mediated by eukaryotic initiation factor 4B. J. Biol. Chem. **261**:11466–11470.
23. **Ray, B. K., T. G. Lawson, J. C. Kramer, M. H. Cladaras, J. A. Grifo, R. D. Abramson, W. C. Merrick, and R. E. Thach.** 1985. ATP-dependent unwinding of messenger RNA structure by eukaryotic initiation factors. J. Biol. Chem. **260**:7651–7658.
24. **Rhoads, R. E.** 1988. Cap recognition and the entry of mRNA into the protein synthesis initiation cycle. Trends Biochem. Sci. **13**:52–56.
25. **Rozen, F., I. Edery, K. Meerovitch, T. E. Dever, W. C. Merrick, and N. Sonenberg.** 1990. Bidirectional RNA helicase activity of eucaryotic translation initiation factors 4A and 4F. Mol. Cell. Biol. **10**:1134–1144.
26. **Sonenberg, N.** 1988. Cap binding proteins of eukaryotic messenger RNA: functions in initiation and control of translation. Prog. Nucleic Acid Res. Mol. Biol. **35**:174–207.
27. **Sonenberg, N.** 1990. Poliovirus translation. Curr. Top. Microbiol. Immunol. **161**:23–47.
28. **Sonenberg, N., M. A. Morgan, W. C. Merrick, and A. J. Shatkin.** 1978. A polypeptide in eukaryotic initiation factors that crosslinks specifically to the 5′-terminal cap in mRNA. Proc. Natl. Acad. Sci. USA **75**:4843–4847.
29. **Wiekowski, M., M. W. Schwarz, and H. Stahl.** 1988. Simian virus 40 large T antigen DNA helicase: characterization of the ATPase-dependent DNA unwinding activity and its substrate requirements. J. Biol. Chem. **263**:436–442.