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Starting at the Beginning, Middle, and End: Translation Initiation in Eukaryotes

Review

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Introduction

In both prokaryotes and eukaryotes, ribosomes are recruited to mRNAs in a sequential, multistep process. In eukaryotes, following the recruitment of the small ribosomal subunit to the mRNA, the mRNA sequence is scanned and the small subunit is placed at the initiation codon. After this, the joining of the large ribosomal subunit to the mRNA completes the assembly of the ribosome. In spite of the similarities, there are large differences between prokaryotes and eukaryotes in how these essential features are enacted. In particular, while the recruitment of the small ribosomal subunit to mRNA in prokaryotes is primarily directed by the basepairing between the 16S rRNA and the Shine-Dalgarno sequence on mRNA, the recruitment of the small ribosomal subunit to mRNA in eukaryotes is primarily directed by protein–protein and protein–RNA interactions.

The recruitment of the 43S small ribosomal subunit complex (i.e., the 40S subunit and its associated initiation factors) to eukaryotic mRNA has historically been depicted as resulting from interactions between a limited set of translation initiation factors (reviewed by Hershey et al., 1996). One of these factors, eIF3, is a 40S subunit–associated factor comprised of at least 8 subunits in mammalian cells that interacts with the mRNA-associated initiation factor eIF4F. As a result, much past and recent work has focused on the interactions of eIF4F with mRNA and eIF3 (Figure 1A).

eIF4F in all eukaryotic cells consists of two core subunits. These are the mRNA cap binding protein eIF4E and the large subunit eIF4G. Recent work on eIF4G has revealed that it contacts eIF3 via its C-terminal domain, while its N-terminal domain is responsible for its interaction with eIF4E (Lamphear et al., 1995; Mader et al., 1995; Ohlmann et al., 1996). The modular nature of eIF4G has allowed for the formulation of a working model by which small ribosomal subunits are recruited to mRNA via the simultaneous association of eIF4G with both eIF4E and eIF3.

Current models of how eIF4F is bound to the mRNA focus on the interaction of its eIF4E subunit with the mRNA cap structure. However, studies on the mechanisms by which an internal ribosome entry site (IRES) and the poly(A) tail on mRNA stimulate 40S subunit recruitment suggest that there are alternative ways to recruit the 43S complex to the mRNA. As a result of these studies, which are covered in more detail below, these current models fail to fully explain this early stage of translation initiation. This review will discuss recent findings that implicate different ways by which the 40S small ribosomal subunit can be recruited to eukaryotic mRNA and the various ways this recruitment step can be regulated. It will then present an integrated model of the 40S subunit recruitment step.

Starting at the Beginning: The Cap Structure and 40S Subunit Recruitment

All eukaryotic mRNAs and many viral RNAs bear a 5′ terminal nuclear modification, the cap structure (^{7}mGpppN). The cap integrates several important functions and affects RNA splicing, transport, stabilization, and translation. The cap structure recruits the small ribosomal subunit (40S subunit) to the mRNA during translation initiation. It may also position the 40S subunits recruited by the mRNA's poly(A) tail (see below) to the very 5′ end of the mRNA. These activities of the cap structure are dependent upon the eIF4F complex, which binds to the cap structure through the cap binding protein eIF4E.

Since uncapped mRNAs can be translated in cell-free extracts (Ohlmann et al., 1995) and uncapped functional mRNAs can be generated in vivo by RNA polymerase III (Gunnery and Mathews, 1995), there is no absolute cap requirement for translation. However, several independent lines of evidence underscore the importance of the cap structure for translation: it profoundly stimulates protein synthesis in vitro as well as in injected oocytes and electroporated cells, and destroying the eIF4F complex or preventing its assembly (see below) inhibits translation initiation.

While the existence of a bridge between the cap structure and the 40S subunit created by an interaction between eIF4F and eIF3 is supported by biochemical evidence, different models have been advanced for the kinetic order of its assembly. In particular, it has not yet been resolved whether eIF4G primarily associates with eIF4E and the cap followed by the association of a eIF3/40S complex, or whether eIF4G first joins the ribosomal complex and subsequently encounters the mRNA with eIF4E bound to the cap (Jaramillo et al., 1991; Joshi et al., 1994). For the sake of clarity, this review will treat the eIF4F–eIF3 interaction as the second step in the initiation cycle (Figure 1A).

In addition, eIF4A is required for cap-stimulated 40S subunit recruitment (Pause et al., 1994a). In higher eukaryotes eIF4A can bind to eIF4G (Lamphear et al., 1995), which thus seems to function as an assembly platform during the early phase of translation initiation (Hentze, 1997). In biochemical assays, mammalian eIF4A displays ATP-dependent RNA helicase activity that is stimulated by the presence of the RNA-binding protein eIF4B (although the two proteins do not seem to interact directly) (Rozen et al., 1990). The precise roles of eIF4A and eIF4B in 40S subunit recruitment still await definition, but most models suggest that they unwind the mRNA in preparation for 40S subunit association (reviewed by Merrick and Hershey, 1996). Because eIF4A

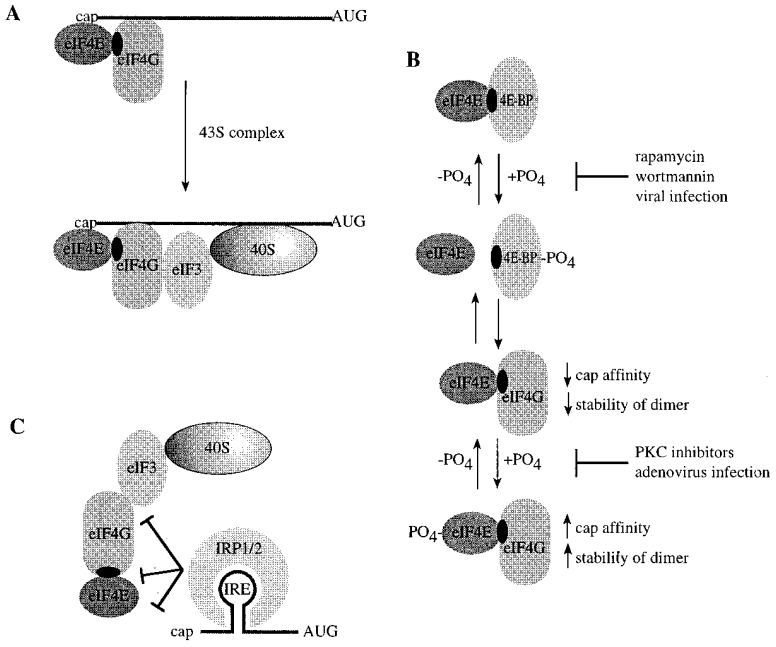

Figure 1. Cap-Stimulated Translation Initiation

(A) eIF4E recruits the 40S subunit to the mRNA via a network of protein interactions. Note that the subunit composition of eIF3 and the 40S subunit are not shown, and that the relative sizes of the proteins are not drawn to scale.

(B) Phosphorylation regulates the activity of the eIF4E/eIF4G complex. The effects of phosphorylation on the association of 4E-BPs with eIF4E, and on the affinity of eIF4E for eIF4G and the cap structure are shown. The location of the inhibitory effects of various drugs and of viral infections on this regulatory circuit are indicated.

(C) Possible mechanisms by which the iron regulatory proteins (IRP1/2), when bound to the iron responsive element (IRE), could block 40S subunit binding are indicated.

does not copurify with eIF4E in lower eukaryotes, this review will not consider it to be an integral subunit of the eIF4F complex, and as a result it will not be represented in the figures.

Knowledge of eIF4F's interactions with the cap structure permits an understanding of cellular and viral strategies to control cap-stimulated translation. For instance, cells express a small family of inhibitory proteins that regulate eIF4F assembly by preventing the association of eIF4E with eIF4G. These are called the 4E-binding proteins (4E-BPs) (Pause et al., 1994b). The 4E-BPs share an amino acid motif (RIIYDRKFLMEC in 4E-BP1) with the N-terminal domain of eIF4G (KKRYDREFLLGF, identical amino acids underlined), which is known to be required for eIF4G's interaction with eIF4E. In their nonphosphorylated form, the 4E-BPs act as competitive inhibitors of the eIF4G–eIF4E interaction, presumably by binding to eIF4E via a region containing this conserved sequence of residues (reviewed by Sonenberg, 1996) (Figure 1B).

Viral infection can induce dephosphorylation of 4E-BPs to enhance their association with eIF4E and thereby inhibit translation (Gingras et al., 1996). Growth factors can induce the phosphorylation of 4E-BP1 to induce its dissociation from eIF4E, thereby to activate translation (Figure 1B). Recent work from several groups has identified some of the critical links in the transduction chain of the growth factor signal to 4E-BP1, the best studied member of the 4E-BPs. Although the mitogen-activated protein (MAP) kinase ERK1 was found to phosphorylate 4E-BP1 on the major site Ser-64 in vitro, and some growth factors that induce Ser-64 phosphorylation activate MAP kinase activities, this family of protein kinases—contrary to initial expectations—does not appear to be involved (reviewed by Sonenberg, 1996). The potent inhibitory effect of rapamycin (which does not inhibit the MAP kinase pathway) on the serum-induced phosphorylation of 4E-BP1 strongly implicated the FRAP/RAFT1 family of kinases to lie within the activation pathway (Lin et al., 1995; Beretta et al., 1996). Early signaling upstream from FRAP/RAFT1 involves phosphatidylinositol-3-kinase (PI3K), because the propagation of the growth factor signal to 4E-BP1 is also prevented by the PI3K inhibitor wortmannin (Figure 1B; von Manteuffel et al., 1996). Future experiments will be geared toward completing the signal transduction pathway from the growth factor signal to the translational apparatus and toward defining the biological roles that the different 4E-BPs play.

Phosphorylation of eIF4E itself appears to enhance its binding to the cap structure and its interaction with eIF4G, and this phosphorylation can be regulated in vivo (Figure 1B). Phosphorylated eIF4E is highly enriched in ribosomes bound to mRNA (Joshi-Barve et al., 1990), possibly because the phosphorylated form of eIF4E associates more readily with the cap structure and eIF4G (Morley et al., 1993). Ser-209 represents the major eIF4E phosphorylation site, but the physiological eIF4E kinase(s) has not yet been identified. Decreasing eIF4E phosphorylation is another viral strategy to reduce host cell mRNA translation: in cells infected with adenovirus, underphosphorylated eIF4E accumulates as host cell protein synthesis declines, while the adenoviral mRNA continues to be translated efficiently since its tripartite leader sequence allows preferential translation at low concentrations of active eIF4E (Huang and Schneider, 1991).

In addition to translational regulation via the general initiation factors 4E and 4G, the translation of specific mRNAs can be controlled via cap-proximal mRNA regulatory sequences. A well-studied example of this is the 5′ terminal oligopyrimidine tract (5′ TOP), which mediates the growth-dependent translational stimulation of a family of mRNAs encoding several ribosomal proteins, the eukaryotic translation elongation factors 1A and 2, and the poly(A)-binding protein (reviewed in Meyuhas

et al., 1996). The 5′ TOP has to follow immediately after the 5′ cap structure. Even the identity of the nucleotide immediately adjacent to the cap may be important for TOP activity since its substitution with a purine abolishes translational regulation (Avni et al., 1994). A key to the understanding of the function of the 5′ TOP should come from the identification and cloning of the responsible *trans*-acting regulatory factor(s).

The *trans*-acting factors responsible for the translational regulation by iron-responsive elements (IREs), which were first defined within the cap-proximal region of ferritin mRNAs, have been identified and intensively characterized (reviewed by Hentze and Kühn, 1996; Rouault et al., 1996). The high affinity binding of iron regulatory protein (IRP)-1 or IRP-2 to the IRE inhibits cap-stimulated translation by blocking the recruitment of the 40S ribosomal subunit to the mRNA (Gray and Hentze, 1994). This inhibitory effect of IRPs bound to the IRE is position dependent and requires the proximity of the IRE to within <60 nucleotides of the cap structure (Goossen and Hentze, 1992). These cap proximal IRE/IRP complexes appear to act as steric "gate keepers": high affinity RNA–protein complexes formed by the bacteriophage MS2 coat protein or the spliceosomal protein U1A with their respective RNA binding sites can exert similar inhibitory effects on the cap-stimulated recruitment of the 40S ribosomal subunit when introduced into the same cap proximal position as the IRE (Gray and Hentze, 1994; Stripecke et al., 1994). These results imply that other translationally regulated mRNAs could exploit a similar mechanism. An important issue that remains to be elucidated is which bridging interaction between the cap structure and the 40S subunits is disrupted by these cap-proximal repressor complexes (Figure 1C).

Starting in the Middle: IRES Elements and 40S Subunit Recruitment

It has long been known that positive-stranded picornaviral mRNAs, whose 5′-noncoding regions (5′ NCRs) range from 650 to 1300 nucleotides and are burdened with many AUG codons and secondary structures, can be efficiently translated in infected cells (reviewed by Meerovitch and Sonenberg, 1993). In addition, picornavirus infection usually leads to a specific inhibition of host cell translation (reviewed by Sonenberg, 1990). These observations indicated that picornaviral mRNAs are translated by a mechanism different from the cap-stimulated scanning mechanism used by most host cell mRNAs. In 1988, both Sonenberg's and Wimmer's groups tested the possibility that ribosomes do not linearly traverse the picornaviral 5′ NCRs to find the start site AUG codon, located hundreds of nucleotides from the uncapped 5′ end of the mRNA. These studies monitored translation of dicistronic mRNAs that contained the 5′ NCRs of poliovirus (Pelletier and Sonenberg, 1988) or encephalomyocarditis (EMC) virus (Jang et al., 1988) inserted between the two coding regions. These dicistronic mRNAs efficiently directed the translation of the first cistron regardless of the sequence in the intercistronic region. However, the second cistrons were only translated when preceded by the viral 5′ NCRs. Subsequent studies have delineated the sequence elements in the viral 5′ NCRs that are sufficient for directing translation of the second cistrons, now known as IRES elements (reviewed by Jackson and Kaminski, 1995).

Subsequently, all picornaviral mRNAs were found to contain IRES elements in their 5′ NCRs. On the basis of their primary sequences, predicted secondary structures, and requirements for efficient translational initiation, the picornaviral IRES elements have been assigned to three groups: the enterovirus and rhinovirus group (type I IRES, e.g. poliovirus), the cardio- and aphtovirus group (type II IRES, e.g. EMCV), and the hepatitis A virus IRES (Jackson and Kaminski, 1995). Within these categories, except for the presence of a pyrimidine-rich sequence element near the 3′ end of each IRES, there is modest conservation of primary sequence and more significant conservation of predicted secondary structure. The main difference between the different classes of picornaviral IRES elements is the location of the IRES with respect to the start site AUG codon. Type I IRES elements can be as far as 50–100 nucleotides upstream of the initiation codon, while type II IRESs, as well as the hepatitis A IRES, include the initiation codon at their 3′ boundary. As a consequence, translational initiation in type I IRESs is at least a two-step process: binding of the 40S ribosomal subunit to the IRES and subsequent relocation of the subunit to the start site AUG codon. The relocation step probably follows a scanning mechanism, because insertion of an AUG codon between the IRES and the initiator AUG designates the new AUG triplet as the start codon (Pestova et al., 1994). In contrast, type II IRES elements are thought to bind ribosomal subunits directly at the start site AUG codon (Pestova et al., 1996a) (Figure 2A).

How prevalent are IRES elements in nonpicornaviral

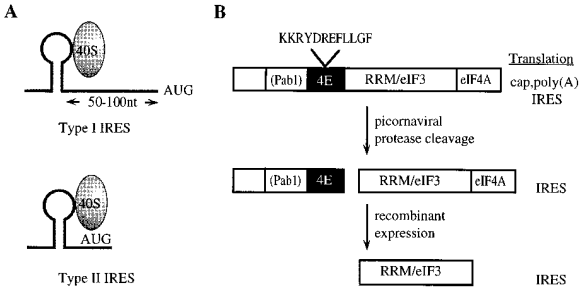

Figure 2. IRES-Stimulated Translation Initiation

(A) Type I IRES elements require the 40S subunit, once bound to the IRES, to scan the mRNA to identify the initiator codon (AUG), while type II IRES elements contain an initiator codon near them. The interaction between the 40S subunit and the mRNA need not be direct. Instead, it could be mediated by bridging factors.

(B) Domain organization and translational capacity of mammalian eIF4G. The relative locations of the eIF4E binding site, the eIF3 binding site, and the eIF4A binding site are shown. The putative RNA-binding site (RRM) and Pab1p binding site, which has been identified in the S. cerevisiae eIF4G, are also shown. The sequence of the amino acid motif in eIF4G, which is required for interaction with eIF4E, is indicated above the eIF4E binding domain. The positions of the eIF4G cleavage site by picornaviral proteases and the eIF4G fragment shown to be required for IRES-stimulated 40S subunit binding are indicated. The ability of each protein to stimulate cap, poly(A) tail (in yeast extracts), or IRES-stimulated translation is also indicated. See text for details.

and cellular mRNAs? To date, a few other viral and cellular mRNAs (reviewed by Jackson and Kaminski, 1995) have been detected that contain IRES elements. Notably, hepatitis C virus (HCV) contains an IRES (Tsukiyama-Kohara et al., 1992; Wang et al., 1993) that extends into its coding region (Reynolds et al., 1995). Several cellular mRNAs that contain IRES elements have been identified (reviewed by Iizuka et al., 1995). Those include mRNAs encoding the immunoglobulin heavy chain binding protein Bip (Macejak and Sarnow, 1991), the Drosophila Antennapedia protein (Oh et al., 1992), and the fibroblast growth factor 2 protein (Vagner et al., 1995). Interestingly, the mRNA that encodes human eIF4G also contains an IRES in its 5′ NCR (Gan and Rhoads, 1996). Although these cellular IRES elements have no obvious sequence or structural similarity to each other or to the well-studied picornavirus IRES elements, the future identification of more cellular IRES elements may reveal a pattern that is obscure so far.

Type I and II IRES elements display different efficiencies in directing translation by internal initiation in vitro (Borman et al., 1995) and in vivo (Borman et al., 1997b). Type I IRES elements generally function poorly in standard cell-free systems and vary greatly in their efficiencies in cell lines of different origins. Interestingly, in some cases the presence of virus-encoded proteases that cleave eIF4G (see below) can overcome the inefficiency of an IRES in a specific cell type (Borman et al., 1997b). On the other hand, type II IRES elements function efficiently in vitro and in a variety of different cell types. The hepatitis A virus IRES is inefficient in directing internal initiation both in vitro and in vivo; this correlates well with the poor growth of the virus in hepatocytes. These findings suggest either that similar *trans*-acting factors mediate IRES usage with vastly different efficiencies, or that different *trans*-acting factors are involved in the modulation of various picornaviral IRES elements, as discussed below. Because picornaviral IRES elements function in the absence of any virus-encoded proteins, the host cell translation apparatus must be capable of performing internal initiation.

How do IRES elements recruit ribosomal subunits? Ribosomal subunits could first bind at or near the 5′ end of the mRNA and could then subsequently be transferred to the IRES, or IRES elements could bind ribosomal subunits directly. Because the type II EMC virus IRES can direct the translation of a circular RNA (Chen and Sarnow, 1995), it is clear that at least this IRES element recruits ribosomal subunits independently of a free 5′ end in the mRNA. It is likely that both RNA–RNA and protein–RNA complexes are involved in the recruitment process. Studies performed with poliovirus mutants have suggested that the location of a pyrimidine-rich tract approximately 24 nucleotides upstream of the start-site AUG codon is an important element of the poliovirus IRES (Pilipenko et al., 1992). Both the pyrimidine-rich sequence element and sequences surrounding the start-site AUG codon have been predicted to be complementary to 18S rRNA (Pilipenko et al., 1992). Of course, it is very difficult to test whether predicted Shine-Dalgarno-like rRNA–polioviral IRES interactions may assist in the recruitment of ribosomes to the IRES. On the other hand, much work has concentrated on the identification of factors that are involved in internal initiation.

Nearly the same set of canonical factors that are important in cap-stimulated translation initiation have been found to be important in IRES-mediated internal initiation (Anthony and Merrick, 1991; Pause et al., 1994a). Recently, Hellen and coworkers provided insights into the mechanism by which eIF4F could be involved in recruitment of ribosomal subunits to the type II EMC virus IRES (Pestova et al., 1996a, 1996b). These studies revealed that eIF4F could bind directly to the IRES element. Evidence was presented that the central third domain of eIF4G, which contains a putative RNA-binding site and the eIF3 binding site, was sufficient to recruit 40S subunits to the IRES. If it turns out that eIF4G contains no other binding sites in the EMCV RNA, then these data suggested that the central third domain of eIF4G binds the EMCV IRES, and once bound, recruits eIF3 and perhaps eIF4A as a means to stimulate ribosome binding (Figure 2B).

That fragmented eIF4G, bound to both eIF4A and eIF3, is important for mediating internal initiation is known from studying several picornaviral proteases that cleave eIF4G to yield an N-terminal eIF4G/eIF4E complex and a C-terminal eIF4G/eIF4A-eIF3 complex (Lamphear et al., 1995; Ziegler et al., 1995a, 1995b) (Figure 2B). The C-terminal fragment of eIF4G with its associated factors has been shown to stimulate the translation of certain viral IRESs (Ohlmann et al., 1996) and, curiously, uncapped mRNAs (Borman et al., 1997a). The cleavage of eIF4G by these proteases serves to enhance viral mRNA expression directed by their IRES elements while at the same time repressing host cell cap-stimulated translation.

Noncanonical translation initiation factors have been hypothesized to be involved in IRES-mediated translation, because none of the known IRES elements functions in the wheat germ extract that is able to mediate cap-dependent translation. Although several *trans*-acting factors have been identified that bind to various IRES elements (reviewed by Belsham and Sonenberg, 1996; Jackson and Kaminski, 1995), the La autoantigen and the poly-pyrimidine tract binding protein, PTB, have received the most attention. Addition of La to rabbit recticulocyte lysates (RRL) greatly enhances the translation of polioviral mRNAs at the correct initiation site and inhibits initiation at incorrect sites. However, the concentration of recombinant La that is needed to accomplish this effect is approximately 10-fold higher than the concentration of La that is present in a Hela extract capable of perfoming the same enhancement when added to the RRL (reviewed by Belsham and Sonenberg, 1996). It has been argued that high levels of recombinant La were needed because La was misfolded, not properly modified or not associated with important auxiliar factors (reviewed by Belsham and Sonenberg, 1996). Clearly, what is needed in these studies is a source of active La. Properly folded recombinant La or La-protein complexes, isolated from mammalian cells, could be isolated and used in cell-free translation assays. Similarly, a general role of PTB in internal initiation is far from being clear. The specific binding of PTB to several IRES elements has not been correlated with a general

role in internal initiation. It has recently been suggested that PTB could enhance the folding of certain IRES elements into active structures by acting as an RNA chaperone. The RNA chaperone property of PTB could then increase the population of properly folded IRES elements that are active in the recruitment of ternary complexes.

Another noncanonical translation initiation factor may have been identified recently by Gamarnik and Andino (1996). These workers monitored the translational efficiency of the poliovirus IRES after microinjection into Xenopus oocytes. They found that the viral IRES did not function in oocytes unless a 300 kDa factor, termed poliovirus translation factor, or PTF, was isolated from human HeLa cells and coinjected with it. PTF was not contaminated by the La protein. PTF was also found to be present in rabbit reticulocyte lysates RRL (though 10× less active), which only poorly supports the function of the poliovirus IRES. Whether the lower activity of PTF in the RRL is responsible for the low translational activation remains to be seen. Although particular factors can enhance the efficiency of certain IRES elements, there is yet no evidence for the requirement in internal initiation of a single noncanonical translation initiation factor.

Why have IRES elements evolved as an alternative means to stimulate 40S subunit binding to mRNA? In the case of picornaviruses, they provide a means of translating the viral mRNA without interfering with RNA elements at the very 5′ end that might be necessary for viral replication. Viral IRESs can also allow for viral mRNA translation under conditions where host cell cap-stimulated translation is shut off. IRES elements on cellular mRNA may have similar functions. For instance, highly structured 5′ NCRs on some mRNAs may have specific functions, such as mRNA localization, and their IRES elements would allow for their continued expression without affecting their cellular location. Similarly, under conditions where eIF4F is inactivated due to cellular heat shock, growth arrest, or position in the cell cycle (reviewed by Rhoads, 1993), cellular mRNA IRESs would allow for their continued expression.

Starting at the End: Poly(A) Tails and 40S Subunit Recruitment

Since its discovery on eukaryotic mRNA over 25 years ago, the function of the poly(A) tail in mRNA translation has been the subject of intensive research (reviewed by Jacobson, 1996). A series of independent experiments performed during the late 1980s and early 1990s led to the conclusion that the poly(A) tail was capable of stimulating the translation of mRNA. First, it was shown that the addition of the poly(A) tail to mRNA stimulated its translation in the amphibian oocyte. In particular, it was shown that blocking the addition of the poly(A) tail to the mRNA, either by mutating the polyadenylation signal on the mRNA or by chemically modifying the 3′ end of the mRNA so that it could not be polyadenylated, resulted in the inhibition of that mRNA's translation. Secondly, a large set of mRNA electroporation experiments revealed that the poly(A) tail acted as an enhancer of mRNA translation, and that the poly(A) tail could act synergistically with the cap structure to stimulate the mRNA's translation. Finally, genetic experiments in the yeast Saccharomyces cerevisiae revealed that the poly(A) tail binding protein, Pab1p, was required for efficient mRNA translation. Similarly, it was shown that the in vitro translation of mRNA was inhibited by the addition of excess poly(A) to the reaction mixture and that this inhibition was relieved by the addition of purified Pab1p.

Subsequently, it was shown that the approximately 2-fold stimulation of mRNA translation by the poly(A) tail in rabbit reticulocyte lysates was due to a stimulation of the joining of the 60S large ribosomal subunit to the mRNA (Munroe and Jacobson, 1990). This work was consistent with the simultaneous findings that many bypass suppressor mutations of a *PAB1* deficiency in yeast also resulted in alterations of the 60S ribosomal subunit (Sachs and Davis, 1989). This combination of in vivo and in vitro data led to the working hypothesis that the poly(A) tail stimulated an mRNA's translation by enhancing the 60S subunit joining step of the initiation pathway.

Although these early genetic and biochemical experiments on the mechanism of poly(A) tail enhancement of translation support the hypothesis that the poly(A) tail was utilized during translation initiation, further progress in this area was hampered by the lack of an in vitro translation system that required the mRNA be polyadenylated for it to be translated. This hurdle was overcome in 1994 with the report of a method of preparation of yeast translation extracts that showed significant stimulation of mRNA translation if the mRNA was capped or polyadenylated (Iizuka et al., 1994). Importantly, the stimulation by these two structures was shown to result from a stimulation of translation and not a stabilization of the mRNA. Furthermore, it was shown that the previously reported synergism between the cap and the poly(A) tail observed in mRNA electroporation experiments was also observed in the in vitro system. One intriguing result from this work that could not be explained by the existing models of translation initiation was how the poly(A) tail on mRNA, in the absence of the cap structure, was capable of stimulating the translation of the mRNA.

Using monoclonal antibodies to Pab1p, it was found that the stimulation of translation by the poly(A) tail in the yeast extracts required Pab1p (Tarun and Sachs, 1995). These data were consistent with the earlier genetic studies that also implicated Pab1p in the translation process. Furthermore, it was shown that the ability of the poly(A) tail and Pab1p to stimulate translation did not require the presence of a functional cap-binding protein (eIF4E). However, the synergistic stimulation of translation by the cap and the poly(A) tail did require the presence of both Pab1p and eIF4E. This suggested that poly(A) tails did not stimulate translation by binding directly to eIF4E, but that the synergistic stimulation of translation required both factors. Finally, it was shown in this report that the poly(A) tail stimulated mRNA translation by enhancing the binding of the 40S small ribosomal subunit to the mRNA.

Thus, both 40S binding and 60S joining had been implicated as the target of poly(A) tails in translation. To reconcile these differences, it was suggested (Tarun and

Sachs, 1995) that the ability of the poly(A) tail to stimulate 40S binding in the reticulocyte lysate was in some way masked, and as a result the involvement of the poly(A) tail in a later initiation step was able to be studied. It was also suggested that the reason alterations in the 60S subunit led to the ability of cells to live in the absence of *PAB1* was because these alterations indirectly led to an increase in the amounts of free 40S subunits. By mass action this would lead to enhanced rates of 40S binding, and thereby perhaps overcome the deficiency in the stimulation of this step resulting from the absence of Pab1p.

How the poly(A) tails could stimulate the binding of the 40S subunit to the mRNA in the yeast system has recently been described (Tarun and Sachs, 1996). Based on the observation that Pab1p was required for the poly(A) tails to function in translation, and that 40S subunit binding was known to be stimulated by the translation initiation factor eIF4F, the potential copurification of Pab1p with eIF4F was investigated. Using classical ^{7m}GDP-affinity chromatography, it was found that Pab1p was indeed associated with eIF4F. Furthermore, it was found that Pab1p's primary contact with the eIF4F complex was through eIF4G, and that this contact required the presence of RNA. By utilizing recombinant fragments of eIF4G and full-length Pab1p, it was found that a 114–amino acid fragment of the yeast eIF4G homolog Tif4632p was capable of binding to Pab1p only when it was bound to poly(A). This fragment was located just N-terminal to Tif4632p's eIF4E binding site. The observation that Pab1p physically associated with eIF4F strongly suggested that the mechanism of 40S subunit recruitment by the poly(A) tail resulted from this interaction.

Evidence that the physical interaction between Pab1p and eIF4G resulted in the recruitment of the 40S subunit to mRNA in vitro was obtained by analyzing yeast extracts containing mutated eIF4G proteins (S. Tarun and A. B. Sachs, submitted). In these experiments, the Pab1p binding site on eIF4G was either partially or completely destroyed by directed mutagenesis. These mutant extracts exhibited little or no ability to be stimulated by the poly(A) tail on mRNA. In contrast, the ability of the cap structure on mRNA to stimulate translation in the extracts was not destroyed. These data, in combination with the other experiments described above, led to the formulation of the model that the poly(A) tail on mRNA stimulates the recruitment of the 40S subunit during translation via the interaction of Pab1p with eIF4G (Figure 3). Due to the simultaneous binding of eIF4E to eIF4G, it was also suggested that the translational synergism between the cap and the poly(A) tail resulted from their binding to a common target, eIF4G. Finally, it was hypothesized that mRNA could be circularized by the interaction of Pab1p and eIF4E with eIF4G.

Although the functional interaction of Pab1p with eIF4G can explain much of the earlier experiments studying the mechanisms by which poly(A) tails stimulate translation, several more issues remain to be resolved. For instance, a direct demonstration that this interaction can allow for mRNA circularization has not yet been obtained. Furthermore, the interaction of Pab1p with other translation initiation factors cannot be discounted at this time. In addition, how poly(A) tails stimulate translation initiation in the absence of the cap structure remains unanswered. Finally, and most importantly, evidence for a similar functional interaction between Pab1p and eIF4G from higher eukaryotes has not yet been reported. As a result, ours and other models about how poly(A) tails stimulate translation in all eukaryotes must be viewed conservatively.

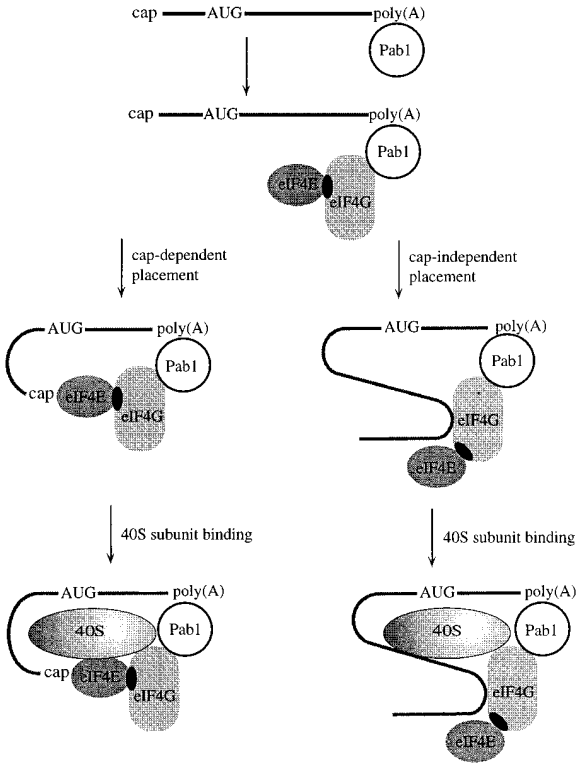

Figure 3. Poly(A)-Stimulated Translation Initiation and a Revised Model for the Mechanism of 40S Subunit Binding to mRNA

Pab1p binds to the eIF4G/eIF4E complex, and this ultimately leads to the stimulation of 40S subunit binding. For cap-stimulated translation, the association of eIF4E with the cap structure could lead to transient or stable mRNA circularization and placement of the 40S subunit near the 5′ end of the mRNA. For cap-independent translation, binding of eIF4G to an IRES (not indicated) would lead to placement of the 40S subunit at a unique position within the mRNA. Binding of Pab1p to eIF4G is shown to be the first step in the assembly of the initiation complex. However, the order of assembly of the final complex could be different than that shown here.

In spite of these open questions, new directions for studies on how mRNA translation could be regulated by mRNA 3′ ends have been generated. For instance, it now seems plausible that some 3′ UTR sequences and their binding factors could work by activating or inhibiting some aspect of Pab1p function, which includes contacting eIF4G and possibly circularizing mRNA. Furthermore, by analogy with the recent identification of the 4E-BP family of proteins, it is possible that one or several repressor proteins negatively regulate Pab1p function by blocking its association with eIF4G. Finally, the discovery of another mRNA binding protein involved in the very earliest of steps during the translation initiation cycle should lead to more directed studies on its potential regulation by other cellular factors.

A Revised Model of 40S Subunit Recruitment to mRNA

The exclusive role of the mRNA cap structure in the 40S subunit recruitment process during translation initiation is no longer tenable. The ability of IRES elements and poly(A) tails in yeast to stimulate 40S subunit binding forces a change in this viewpoint. While it is almost certain that the vast majority of mRNAs are translated by 40S subunits scanning from the cap structure, it now seems likely that the 40S subunit can also be brought to the mRNA via an interaction with the mRNA poly(A) tail. Subsequently or simultaneously with this interaction, the cap structure, due to its high affinity for eIF4E, could act as a docking site for the recruited subunit (Figure 3). In this model, both the cap structure and the poly(A) tail share the function of 40S subunit recruitment, while the cap structure has the exclusive role of docking the subunit onto a unique position in the mRNA. We note that the role of poly(A) tails in translation in higher eukaryotes is assumed but not yet shown since only the role of poly(A) tails in yeast translation has been thoroughly studied.

Cellular IRES elements could replace the role of the cap structure on those mRNAs where the cap is either masked or eIF4E is inactive (Figure 3). Recruitment of the 40S subunit to the mRNA by the poly(A) tail could occur prior to or simultaneously with the placement of the subunit at a position on the mRNA determined by the location of the IRES element. In this model, the cap structure and the IRES element have identical functions in the translation process: they assist in recruitment of the 40S subunit to the mRNA, and they provide a loading site for 40S subunits at a unique position on the mRNA.

Future work in this exciting area of translation research should help to test the basic tenets of this model. Since many of the central experiments so far have been carried out in cell-free systems and commonly under conditions where mRNA is limiting, it will be important to determine the interplay of the diferent modes of ribosome recruitment under conditions of mRNA competition for initiation factors. Likewise, we do not yet understand much regarding possible differences between the first and subsequent rounds of initiation, and the roles of the cap, IRES and poly(A) tail in ribosome recycling. Along the way, it is anticipated that new insights into how an mRNA's expression can be controlled in the cytoplasm of eukaryotic cells will be uncovered. With more information, it will hopefully become clearer how mRNA sequences, including the 5′ NCR and the 3′ UTR, can regulate an mRNA's expression.

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