

THE MOLECULAR MECHANICS OF EUKARYOTIC TRANSLATION

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■ **Abstract** Great advances have been made in the past three decades in understanding the molecular mechanics underlying protein synthesis in bacteria, but our understanding of the corresponding events in eukaryotic organisms is only beginning to catch up. In this review we describe the current state of our knowledge and ignorance of the molecular mechanics underlying eukaryotic translation. We discuss the mechanisms conserved across the three kingdoms of life as well as the important divergences that have taken place in the pathway.

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INTRODUCTION

This chapter reviews what we think we know about the molecular mechanisms underlying protein synthesis in eukaryotic organisms and outlines what we do not know. Most molecular biology textbooks contain beautiful cartoons of the current model of the pathway of translation in eukaryotes. These models, albeit useful for teaching purposes and to guide the thinking of researchers, have also lulled many in the biological community into a false sense of the level of our understanding. It is not uncommon to hear, either explicitly or implicitly, the statement that “translation is a solved problem.” Therefore, we examine the current models of the steps of eukaryotic translation to determine where they are soft and where they are firm. The enormity of this task compels us to limit our discussion to the fundamental mechanisms underlying eukaryotic translation. Translation of specific mRNAs, ER-associated translation, translational control, and special cases of translation such as internal ribosome entry, frame-shifting, ribosome shunting, etc., are not discussed, except as an occasional example in which these events have shed light on the underlying mechanics of eukaryotic protein synthesis.

Overview of the Steps of Translation

Translation can be broken into four stages: initiation, elongation, termination, and recycling. In initiation, the ribosome is assembled at the initiation codon in the mRNA with a methionyl initiator tRNA bound (presumably) in its peptidyl (P) site. In elongation, aminoacyl tRNAs enter the acceptor (A) site where decoding takes place. If they are the correct (cognate) tRNA, the ribosome catalyzes the formation of a peptide bond. After the tRNAs and mRNA are translocated such that the next codon is moved into the A site, the process is repeated. Termination takes place when a stop codon is encountered and the finished peptide is released from the ribosome. In the final stage, recycling, the ribosomal subunits are dissociated, releasing the mRNA and deacylated tRNA and setting the stage for another round of initiation.

The above outline of the stages of translation describes the fundamental events in the process that occur throughout all kingdoms of life. Although these fundamental events are the same, how they are achieved in each kingdom sometimes differs. The differences in the processes required to make a protein in each of the three kingdoms may well contain important information about the underlying molecular mechanics of the processes in each kingdom. Therefore, our discussion of each phase of eukaryotic translation begins with a comparison of the steps and components used to accomplish it in each kingdom.

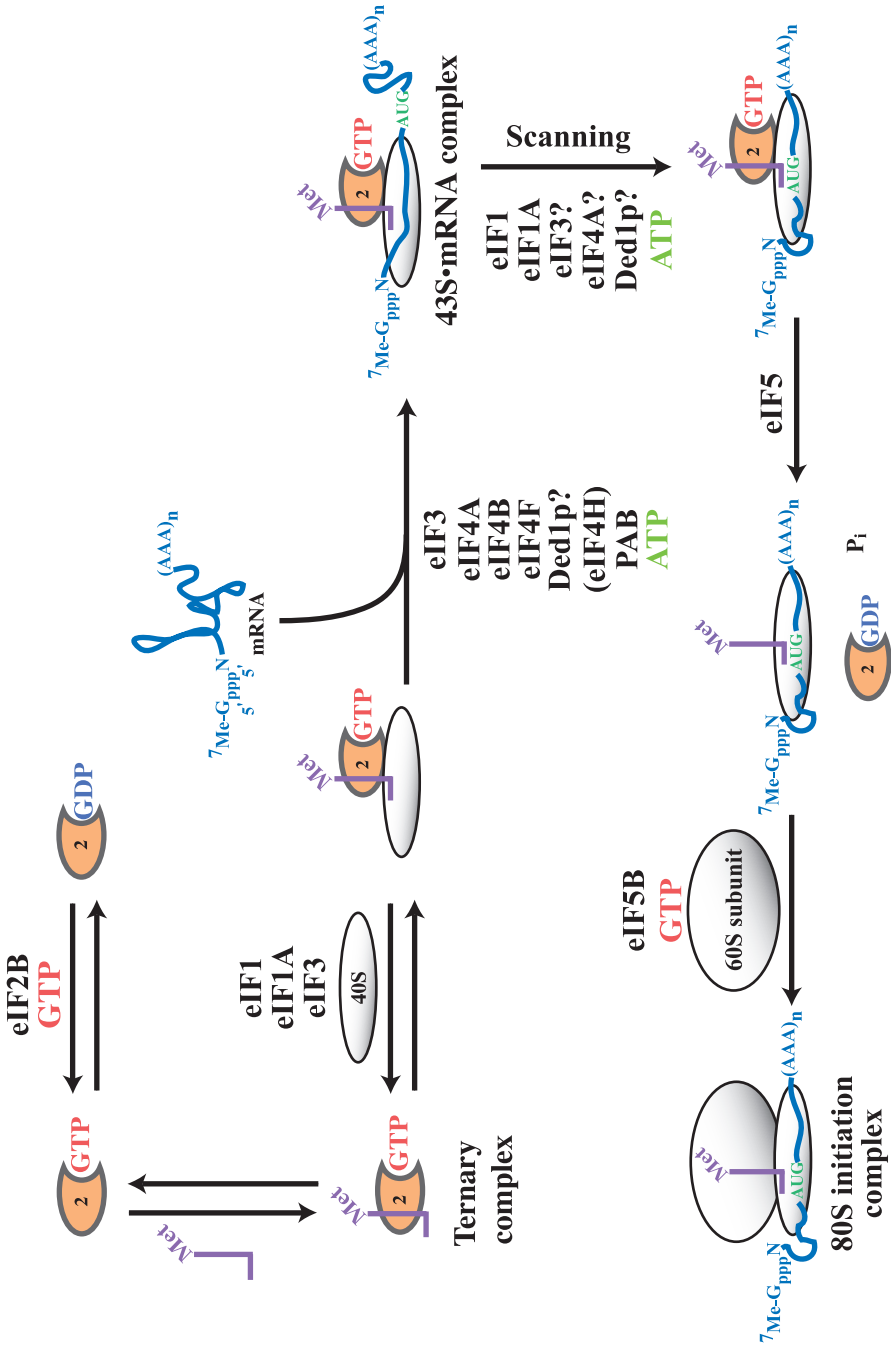
INITIATION

Summary of the Current Model of the Steps of Eukaryotic Translation Initiation

The current model of the steps of eukaryotic translation initiation is shown in Figure 1 (1). The first step in the initiation pathway is the assembly of the eIF2·GTP·Met-tRNA_i ternary complex. Because eIF2 has ~100-fold higher affinity for GDP than for GTP (2–5) and the rate constant for GDP release is slow (2, 6, 7), the eIF2·GDP complex that results from each completed round of translation initiation must be recycled to eIF2·GTP before a new round can begin. This exchange reaction is facilitated by eIF2B. After its formation, the ternary complex binds to the small (40S) ribosomal subunit. This binding is facilitated by (at least) eIFs 1, 1A, and 3. The resulting complex is called the 43S complex. The eIF4F complex assembles on the 5'-cap of the mRNA and unwinds structures found in the 5'-untranslated region (UTR). This is accomplished through the ATP-dependent action of eIF4A assisted by the RNA-binding proteins eIF4B and, in mammals, eIF4H. eIF4F, in conjunction with eIF3 and the poly(A) binding protein (PAB) bound to the 3'-poly(A) tail, loads the mRNA onto the 43S complex. The 43S complex then begins scanning down the message in the 5' to 3' direction, looking for the initiation codon. This scanning process is thought to require ATP hydrolysis, although the ATPase involved has not been identified. eIF1 and eIF1A may play a role in this scanning process as well. When the 43S complex encounters an AUG codon that is embedded in a favorable sequence context (e.g., the Kozak sequence), usually the first AUG, codon-anticodon base pairing takes place between the initiation codon and the initiator tRNA in the ternary complex. This then triggers GTP hydrolysis by eIF2, a reaction facilitated by the GTPase-activating protein (GAP) eIF5. eIF1 also plays a role in the detection of the correct initiation codon. After GTP hydrolysis by eIF2, eIF2·GDP releases the Met-tRNA_i into the P site of the 40S subunit and then dissociates from the complex. In the current model, eIFs 1, 1A, 3, and 5 also dissociate at this stage. At some point either before or after GTP hydrolysis by eIF2, eIF5B·GTP binds to the complex. Once the other factors are gone, it facilitates the joining of the large (60S) ribosomal subunit to the 40S·Met-tRNA_i·mRNA complex. This event triggers GTP hydrolysis by eIF5B, and, because the eIF5B·GDP complex has a low affinity for the ribosome, it dissociates from the complex. This is thought to be the end of translation initiation, although other steps may be required before the complex is fully active to make a peptide bond (8, 9).

Comparative Translation Initiation

BACTERIA Translation initiation in bacteria involves three initiation factors: IF1, IF2 and IF3. IF1 binds over the A site of the small ribosomal subunit (10, 11) and is thought to prevent the initiator tRNA from binding to the A site,



instead promoting its binding to the P site (12). IF2 is a GTPase that enhances binding of the initiator tRNA to the small ribosomal subunit and likely also facilitates the subunit joining step (12–14). IF3 appears to have at least four functions (12): to ensure the fidelity of initiation site selection; along with IF2 to help select the formylated methionyl initiator tRNA (fMet-tRNA_i) for use in initiation rather than the elongator methionyl tRNA used to insert methionine residues into polypeptides during elongation; to dissociate 70S ribosomes into 30S and 50S subunits and likely prevent premature association of the subunits during initiation; and to act during ribosome recycling to remove the deacylated tRNA from the P site of the 30S subunit (15).

The recruitment of the mRNA to the small ribosomal subunit is accomplished in bacteria via a base-pairing interaction between the 3'-end of the 16S rRNA (the anti-Shine-Dalgarno) and the purine-rich Shine-Dalgarno sequence located upstream of the initiation codon in the mRNA. Because the Shine-Dalgarno sequence is usually ~10 bases upstream of the initiation codon, it makes the placement of the initiation codon in or near the P site of the small ribosomal subunit a relatively straightforward process.

EUKARYOTES Although the fundamental goals of translation initiation are the same in bacteria and eukaryotes—to get the initiator tRNA and the mRNA onto the small ribosomal subunit, to find the AUG codon, and to join the ribosomal subunits to form an initiation complex—the steps and machinery required to accomplish these goals are much more complicated in eukaryotes than in bacteria. The free amino group on the methionine moiety attached to the initiator tRNA, which is formylated in bacteria, is free in eukaryotes, although the functional significance of this difference is not yet clear. Perhaps most remarkably, the three translation initiation factors of bacteria are replaced by at least 12 in eukaryotes, which are comprised of at least 23 different polypeptides. Two of the bacterial initiation factors have clear orthologs in eukaryotes (16). The naming of eukaryotic initiation factors is somewhat confusing. The ortholog of bacterial IF1 is not eIF1 (the *e* stands for eukaryotic) but is instead eIF1A, and the ortholog of IF2 is eIF5B, not eIF2 (17–19). At the sequence level, there does not appear to be a clear counterpart to IF3 in eukaryotes. However, based on functional and structural similarities, eIF1 is suggested to be the eukaryotic ortholog of IF3 (20, 21). [Oddly, a few bacteria have a gene encoding a clear eIF1 ortholog, although its function in these organisms is not known (16).]

Functionally, the story is slightly more complex. Mammalian eIF1A has been reported to prevent spurious association of ribosomal subunits (22) just as its ortholog IF1 does. The large, multisubunit factor eIF3 also has antisubunit-

Figure 1 Cartoon of our interpretation of the current model of the steps in eukaryotic translation initiation and the roles of the factors. This is just a model and should not be regarded as factual. For clarity, the 5'- and 3'-ends of the mRNA are not shown interacting.

association activity (23–25) and thus eukaryotes may have multiple means for dissociating prematurely coupled ribosomal subunits and/or preventing their premature association. Although eIF5B is the clear ortholog of bacterial IF2, some of the proposed roles of IF2 appear to be performed in eukaryotes by eIF2 rather than by eIF5B. In its GTP-bound form, eIF2 forms a complex with Met-tRNA_i and carries the initiator tRNA onto the small ribosomal subunit, similar to the initiator tRNA loading function of IF2 in bacteria [although it is still controversial whether IF2 forms a complex with initiator tRNA in bacteria prior to binding to the 30S subunit (26, 27)]. In contrast, eIF5B does not bind initiator tRNA detectably in solution (28; L.K. & J.R.L., unpublished), although it stabilizes initiator tRNA binding to the ribosome (17, 29). eIF5B does, however, play a critical role in facilitating the joining of the two ribosomal subunits following location of the initiation codon (30), just as IF2 is proposed to do (14). Finally, as mentioned above, it is not known whether there is an actual ortholog for IF3 in eukaryotes, but, like IF3, eIF1 is clearly involved in ensuring the fidelity of initiation site selection (21, 31, 32), suggesting they may at least be functional orthologs (21).

The other translation initiation factors in eukaryotes do not appear to have any counterparts in bacteria. eIF2, for which the closest relative in bacteria is the elongation factor EF-Tu (33), functions as previously described to bring the initiator tRNA onto the ribosome. It may also be directly involved in locating the initiation codon (34). eIF3 is a giant heteromultimeric complex comprised of five core subunits in yeast (35) and at least ten subunits in mammals (36–39) and plants (40). With a mol wt of 360 kDa in yeast, it is roughly one quarter of the size of the small ribosomal subunit. The evidence to date suggests a role for eIF3 in facilitating the binding of both the ternary complex and the mRNA to the 40S subunit (38, 41–44). eIF3 also binds to most other initiation factors plus the small ribosomal subunit, suggesting that it may function as a central hub in the assembly of the translation initiation complex. In general, the function of this factor is still not entirely clear, as discussed in more detail below.

Location of the initiation codon in eukaryotes is a very different process from that in bacteria. Although the 18S rRNA of the eukaryotic small ribosomal subunit is very similar to bacterial 16S rRNA over most of its length, the anti-Shine-Dalgarno sequence found in 16S rRNA is missing in 18S rRNA (45). Correspondingly, eukaryotic mRNAs do not have a Shine-Dalgarno sequence that allows easy identification of the initiation codon, but rather have a 5'-7-methylguanosine cap structure that identifies the 5'-end of the message. The 43S complex is proposed to be loaded onto the 5'-end of the mRNA via the cap and to then locate the initiation codon via the scanning process (46). As long as the 43S complex can be faithfully loaded onto the message at its 5'-end and then scan along linearly without hopping about, this method should allow the identification of the correct (usually the first) initiation codon.

The initial loading of the mRNA onto the 43S complex itself presents several problems for the eukaryotic translational apparatus. In bacteria, transcription and

translation are coupled processes and thus as soon as the Shine-Dalgarno sequence emerges from the transcriptional apparatus it can be bound by the small ribosomal subunit, whereas in eukaryotes, the mRNA substrate for translation initiation is thought to be a complete, fully processed messenger RNA, potentially rife with secondary and tertiary structures and coated with RNA-binding proteins such as hnRNPs. [However, the first round of translation initiation on an mRNA may well occur concomitantly with export of the mRNA from the nucleus. Thus, a free, full-length mRNA may not be the substrate for initial initiation (47).] The eIF4 group of initiation factors, which are not present in bacteria, appears to be required because of the uncoupling of transcription and translation and the lack of the Shine-Dalgarno initiation codon identification system in eukaryotes. eIF4A is a DEAD-box RNA-dependent ATPase (48) that can unwind RNA duplexes in vitro and thus has been proposed to be an RNA helicase that functions to unwind regions of secondary and tertiary structure in the 5'-ends of mRNAs. eIF4B is an RNA-binding protein that stimulates the RNA-unwinding activity of eIF4A in vitro (49–52) and in vivo suppresses both mutations in eIF4A (53) and the inhibitory effect of secondary structure in the 5'-untranslated region of a reporter gene (54). It is therefore thought that eIF4B assists eIF4A in unwinding structures in mRNAs. A second RNA-binding protein with similar functions, called eIF4H, has also been identified in mammals (55–57). eIF4E binds to the 7-methylguanosine cap on mRNAs, thus locating their 5'-ends. Both eIF4E and eIF4A bind to eIF4G, which, like eIF3, is thought to act as a multifaceted adapter protein—a sort of central hub for interactions among factors in the initiation apparatus (58). Together, eIFs 4A, 4E, and 4G are called eIF4F (59). The proposed role of the eIF4F complex is to bind to the 5'-end of the mRNA, unwind any structures found there, and then facilitate the loading of the 43S complex onto the now unstructured 5'-untranslated region (5'-UTR). Since the RNA-binding proteins that coat an mRNA probably must also be removed prior to 43S complex loading, it may be that eIF4F and/or eIF4A perform this task as well.

In addition to the 7-methylguanosine cap structure on their 5'-ends, eukaryotic mRNAs have 3'-poly(A) tails that are bound by the poly(A)-binding protein, or PAB. PAB interacts with eIF4G, and this interaction is thought to lead to the circularization of eukaryotic mRNAs, which in turn stimulates translation (60–64). The prevailing notion is that this interaction facilitates binding of the 43S complex to the mRNA, although this is most likely not the whole story (see discussion below). This system provides a quality control mechanism: If the mRNA has been partially degraded and has lost its 3'-end, it will not be translated efficiently. Thus, the system helps guard against the synthesis of truncated proteins that could be toxic to the cell. Translation of degraded mRNAs is somewhat less of a problem in bacteria because of the coupling between transcription and translation—as the 3'-end of the message is being made, ribosomes are rapidly moving down from the 5'-end to translate it. Of course, the presence of tmRNAs and the *trans*-translation system in bacteria that can rescue

ribosomes stuck at the end of partially degraded messages lacking stop codons (65) indicates that 3'-degradation of mRNAs is also important in bacteria. One interesting difference between the two kingdoms in this regard is that it is apparently sufficient for bacteria to deal with the problem after the fact (i.e., release the stalled ribosome and tag the incomplete protein for degradation), whereas eukaryotes have had to develop a system to prevent the translation of truncated mRNAs from happening in the first place.

In addition to the core group of initiation factors, a number of proteins have been suggested to be involved in the initiation process, although their roles, if any, are not yet clear. For example, eIF5A was initially isolated as a protein from rabbit reticulocyte lysates that increased the efficiency of assays measuring the formation of the first peptide bond from 80S initiation complexes (66, 67). Although it is an essential protein in yeast, shutting off expression of an unstable form of eIF5A has only a small (\leq twofold) effect on overall protein synthesis *in vivo*, suggesting it may not in fact be a general translation initiation factor (68). Other data suggest a role for eIF5A in mRNA turnover (69), in ribosome biogenesis, and in the maintenance of cell wall integrity (70). Other potential factors such as eIF6, eIF2A (71), eIF2C (72), and an ATP-binding protein called ABC50 (73) have all been implicated in translation initiation, although eIF6 and eIF2C have also been implicated in other processes, 60S subunit biogenesis (74, 75), and RNA interference (e.g., 76), respectively.

The differences between the required components for eukaryotic and prokaryotic translation might be construed as excess factors required solely for regulatory purposes. However, most of the authentic initiation factors absent in bacteria but present in eukaryotes appear to be involved in central aspects of the mechanism of translation rather than serving peripheral, albeit important, roles. Furthermore, eIFs 1, 1A, 2, 2B, 3, 4A, 4E, 4G, and 5 as well as ded1p are all essential in yeast, further arguing against their being merely ornamentation on top of the core mechanism of bacterial translation. One of the few factors that is not absolutely essential in yeast is eIF5B, the ortholog of bacterial IF2, although its deletion produces a severe slow-growth phenotype (17).

ARCHAEA Translation initiation in archaea appears to constitute an evolutionary rest-stop between the bacterial and eukaryotic systems. Archaeal mRNAs are not capped and most contain Shine-Dalgarno-like sequences upstream of their initiation codons (33). Accordingly, in many cases a prokaryotic-like mode of initiation codon recognition appears to be used in archaea (33), i.e., via base pairing between the 16S rRNA and the Shine-Dalgarno sequence upstream of the initiation codon. This is consistent with the lack of a nucleus in archaea and the presumed coupling between transcription and translation. However, a significant number of archaeal mRNAs lack appropriately spaced Shine-Dalgarno sequences upstream of their initiation codons, and thus other mechanisms of initiation site selection, possibly akin to eukaryotic scanning, may also take place (33, 77).

Like both bacteria and eukaryotes, archaea have IF1/eIF1A and IF2/eIF5B orthologs. [To avoid confusion, we adopt the nomenclature of Dever (20) and call the archaeal factors by the names of their eukaryotic counterparts, for instance, a-eIF5B.] Unlike translation initiation in bacteria, the methionine on the initiator tRNA does not appear to be formylated in archaea (78). The initiator tRNA itself is a mix of eukaryotic and bacterial features; several of the conserved initiator tRNA identity elements are the same as those found in the bacterial initiator tRNA, whereas others, including the important A1:U72 base pair at the end of the acceptor stem, are the same as those found in the eukaryotic tRNA (78). Although all three kingdoms contain IF1/eIF1A counterparts (16, 79), the eukaryotic and archaeal versions are more similar to each other than are the archaeal and bacterial versions (16). In addition, archaea contain an eIF2 ortholog (80) and an eIF1 ortholog (16). They also have proteins related to two of the five subunits of the GDP:GTP-exchange factor for eIF2, eIF2B (81), although it is unlikely that they actually perform this function because the subunits to which they are homologous are not the catalytic subunits of the complex (82). Thus it appears that archaea possess some of the same translation initiation machinery as eukaryotes, albeit in a bare-bones version.

There is probably some significance to which components of the translation initiation machinery are common to eukaryotes and archaea and which are not. First, the core components, IF1/eIF1A, IF2/eIF5B, and IF3/eIF1 (the last being a tenuous homology at this point), must ensure that the initiator tRNA gets into the right place on the small ribosomal subunit, then that a start codon is also in the right place and that subunit joining happens at the right time. Because eIF1 and eIF1A may both interact with the initiator tRNA during initiation, the reason why archaea have more eukaryotic-like versions of these factors may be because the eukaryotic and archaeal initiator tRNAs are more similar than are the archaeal and bacterial tRNAs (e.g., the A1:U72 base pair and the lack of formylation of the methionine). Also, eIF1A and eIF1 may have evolved to work in regions of the small ribosomal subunits of eukaryotes and archaea that are structurally distinct from the same regions in the bacterial 30S subunit; at least in terms of their complements of ribosomal proteins, the archaeal ribosome is more similar to the eukaryotic ribosome than to the bacterial ribosome (83).

Differences between the archaeal and eukaryotic translation initiation apparatus are also potentially informative. For instance, as previously pointed out by Dever (20), it is striking that archaea possess an eIF2 but not an eIF5 ortholog, given that eIF5 is thought to act as a GTPase activating protein (GAP) for eIF2. Thus, a-eIF2 must either be capable of hydrolyzing GTP without assistance from a GAP, or a GAP that is not homologous to eIF5 must exist in archaea. If there is no eIF5 equivalent, this could be because of the different modes of initiation site selection in archaea and eukaryotes, which would in turn suggest that eIF5 plays a direct role in location of the initiation codon during scanning, rather than acting simply as a GAP. The lack of eIF3 in archaea is also striking because eIF3 is thought to be a centrally important factor in eukaryotes, acting as an interaction

hub for most of the components of the machinery and facilitating binding of the eIF2·GTP·Met-tRNA_i ternary complex and mRNA to the small ribosomal subunit. Why would eukaryotes need a very large factor to help binding of the ternary complex to the small ribosomal subunit but archaea would not? Perhaps facilitating ternary complex binding is not actually one of eIF3's essential functions in eukaryotes but rather the consequence of mutual interaction among eIF3, the 40S subunit, and eIF2. If one of eIF3's main roles is to aid in mRNA loading onto the 43S complex and initiation site location, it might then make sense that archaea lack the factor given their apparently different mode of initiation site selection.

By the same logic, it makes some sense that archaea lack the eIF4 complement of factors. It has been suggested, however, that there might in fact be one or more orthologs of eIF4A in archaea (77, 84), although this assignment is based entirely on sequence similarity, and DEAD box proteins in general share significant sequence similarity with eIF4A [the canonical DEAD box protein (85)]. The sequence identity between yeast eIF4A and the putative eIF4A orthologs in archaea is ~36%. For comparison, the sequence identity between yeast eIF4A and Fallp, a nucleolar DEAD box protein involved in ribosome biogenesis and not translation initiation (86), is 57%, and the sequence identity between yeast eIF4A and the 50S-subunit associated protein DbpA from *Escherichia coli* is 34%. Thus an identity of 36% does not imply a common function for DEAD box proteins. It has also been suggested that the *E. coli* cold-shock protein DeaD/CsdA is a bacterial version of eIF4A (87). While this suggestion suffers from the same sequence similarity constraints just discussed, it was strengthened somewhat by data showing that the protein could promote the translation of a structured mRNA *in vitro* (87). This effect, however, did not depend on ATP hydrolysis, as expected for the action of a true eIF4A ortholog. Furthermore, it was later shown that DeaD/CsdA can function in ribosome biogenesis *in vivo* (88), casting some doubt on its role in translation initiation. In general, there is no convincing evidence to date of eIF4A counterparts acting in translation initiation either in archaea or bacteria.

The Order of Events

Much effort has gone into defining the order of the steps in the pathway of eukaryotic translation initiation. An understanding of this order is a necessary foundation for attempts to understand the molecular mechanics of each step in the pathway. Assignment of the order, however, has often been complicated by confusion over the difference between kinetics and thermodynamics. Thermodynamics can tell us nothing about the order of events; this is solely the domain of kinetics. For example, the determination that A binds more tightly to B than C does provides no evidence that A binds to B before C.

There is considerable evidence behind the assignment of the order of several steps. For example, ternary complex clearly must be formed at the beginning of the pathway and bind to the 40S subunit prior to initiation codon location. In

contrast, the evidence supporting the assigned order of other steps is less compelling. For example, in the current model, ternary complex binds to the 40S subunit prior to binding of the mRNA. The assignment of this order of binding does not appear to be strong, however. Although ternary complexes can bind to 40S subunits in the absence of mRNA, small model mRNAs can also bind to naked 40S ribosomal subunits with K_{ds} in the high nM to low μ M range (89; D. Maag & J.R.L., unpublished), suggesting that the ribosome has a functional mRNA-binding site in the absence of ternary complex and other factors. A priori there does not seem to be any reason why unwinding of the mRNA's 5'-UTR and loading of the message onto the 40S subunit by the factors could not take place, at least some of the time, prior to binding of the ternary complex. In fact, on the GCN4 mRNA in yeast, after a short open reading frame upstream of the GCN4 ORF has been translated, the 40S subunit lacking ternary complex appears to scan along the mRNA on its own before rebinding another ternary complex and recommencing initiation (90), consistent with the idea that mRNA can be bound to the 40S subunit in the absence of a ternary complex. A similar model was proposed to explain why lowering the concentration of eIF2 in an in vitro system increased the efficiency with which the second of two AUG codons was used to initiate translation: The 40S subunit without the ternary complex binds mRNA and scans past the first AUG before binding ternary complex and becoming competent to locate the (second) initiation codon (91). Resolution of the order of ternary complex and mRNA binding awaits detailed kinetic analysis of these steps.

The prevailing model regarding the timing of the release of the initiation factors is that eIFs 1, 1A, 3, and 5 are released from the 40S subunit along with eIF2·GDP following initiation codon location. Much of the data for this part of the model comes from experiments in which preinitiation complexes were isolated by centrifugation on density gradients and then the fractions subjected to Western blot analysis to determine whether the factors were still associated with ribosomal complexes. This technique, although somewhat informative about the stability of the factor-ribosome complexes, lacks kinetic and thermodynamic resolution. Even complexes that are very stable on the time scale and/or under the concentrations of translation initiation can dissociate from the preinitiation complexes during the lengthy process of gradient fractionation. Therefore, until a thorough kinetic dissection of the dissociation of the factors from actively initiating ribosomal complexes can be performed, determination of when most of the factors leave the ribosome will remain unresolved.

Complex Complexes: Interactions Among the Factors

Considerable attention has been directed in the last five years toward determining all of the interactions among the components of the initiation machinery. Initially, the degree of separation between any two components in the system—how many intermediate components bridged the interaction between any two factors—appeared to be amazingly small, perhaps only one or two. As more

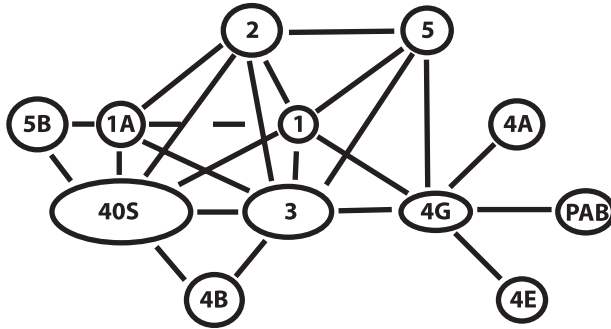


Figure 2 Map of known interactions among eukaryotic translation initiation factors and the 40S ribosomal subunit. The dashed line between eIFs 1 and 1A indicates that this interaction might be indirect (mediated by conformational changes in the 40S subunit).

interactions are discovered and mapped, the real answer may in fact turn out to be closer to zero; that is, everything interacts directly with everything else. As can be seen from Figure 2, not many more interactions need to be found before this becomes true.

THE MULTIFACTOR COMPLEX(ES) Clearly one hub of interactions is eIF3. There are data indicating that one or more of the subunits of eIF3 interact with eIFs 1 (35, 92), 1A (93), 2 (42), 4B (94, 95), 4G (96, 97), and 5 (35, 44, 97), as well as with the 40S subunit (39, 98, 99). In addition, various subunits of eIF3 have been shown to interact with a wide variety of other proteins in the cell, most notably with cytoskeletal proteins (100–104) and components of the proteasome (reviewed in 105). These interactions suggest that eIF3 may function not only as a central organizer of the translation initiation machinery but also as a key link between the initiation machinery and the rest of the cell.

A variety of experiments have shown that a “multifactor complex” (MFC) consisting of at least eIFs 1, 2, 3, 5, and Met-tRNA_i (presumably bound to eIF2) exists stably *in vivo* (106) and that the integrity of this complex is important for translation initiation (42, 92, 97, 106, 107). Thus, at least some components of the translational machinery may function *in vivo* as a unit that binds *en masse* to the 40S ribosomal subunit and the mRNA, coordinately facilitates the loading of the mRNA and location of the initiation codon, and then dissociates from the ribosome as a single complex. This idea is reminiscent of the recent proposal in the splicing field, based on isolation of a similar stable multifactor spliceosomal complex, that most of the components of the spliceosome may function in splicing as one unit, rather than coming on and off the pre-mRNA at different times (108). This stable multifactor arrangement could even be a general feature of how complex biological processes operate within the cell. It would have the advantage that it reduces the number of assembly and disassembly steps required

for each round of the process in question and it ensures that all the required components are always in the same place at the same time. This could be particularly important inside a cell where macromolecular diffusion can be slow (109). Another advantage might be that once the complex was assembled correctly, its constituent parts would be resistant to competing inhibitory interactions, either spurious or regulatory, with other components of the cell [this assumes a slow dissociation rate for the correct components within the complex—which seems to be the case for the MFC (106)].

At least some of the interactions among the factors may be dynamic and only occur at certain points in the initiation pathway. Several observations support this notion. First, some components of the system appear to stay associated with the 40S subunit after other components have dissociated. For example, as described above, eIF2, at least, has most likely left the 40S subunit before eIF5B facilitates subunit joining. Furthermore, in the cell the concentrations of some of the components of the multifactor complex are higher than others, suggesting pools of certain factors that are associated with the complex and other pools that are not. In yeast, careful measurements of the concentrations of most of the factors has revealed that there is approximately threefold more eIF1 than eIF3 and ~50-fold more eIF4A than eIF4G (110), suggesting the existence of pools of eIF1 not associated with the multifactor complex and large pools of eIF4A not associated with eIF4F. It seems unlikely that these pools of factors not associated with multifactor complexes play no roles in initiation. Finally, in several cases, interactions between two factors and a third factor are mutually exclusive, suggesting that if they occur as part of the initiation pathway, they do so at different times. For example, the interactions of eIFs 1 and 5 with eIF4G are mutually exclusive (111), as are the interactions of eIFs 2 and 4G with eIF5 (97) and the interactions of eIFs 5 and 2B with eIF2 (112).

MOVING MOUNTAINS AND LOWERING ENERGY BARRIERS: MODULATING ENERGETICS IN COMPLEX SYSTEMS In addition to colocalizing the factors and coordinating their binding and release, interactions among factors could induce conformational changes that modulate their behavior at different points in the pathway. For example, binding of factor A to factor B could enhance or reduce factor B's affinity for another component of the system. Fundamentally, such changes in thermodynamic and kinetic parameters as the processes proceed from one step to the next allow complex biological systems to operate. Although our knowledge of the physical basis for each of the steps in eukaryotic translation initiation is limited, some studies are suggesting the interconnectedness of the energetics in the system. For example, studies have indicated that eIF4E's affinity for the 7-methylguanosine cap structure is enhanced by interaction between eIF4E and eIF4G, and that the interaction between eIF4G and PAB increases eIF4E's cap affinity still further (113–115). Data also suggest that eIF4A and eIF3 bind to eIF4G cooperatively (116) and since there is no known interaction between

eIF4A and eIF3, this effect could be the result of a conformational change in one or more factors. The same could be true for eIFs 1 and 1A, which bind cooperatively to the 40S subunit (117). These studies are most likely just beginning to elucidate the modulation in the energetics of different interactions that takes place during the course of translation initiation.

COMPLEX STRUCTURES Structures of complexes are needed to make molecular sense of changes in thermodynamic and kinetic parameters that occur from step to step in the initiation process. Progress has been made in the past few years toward understanding the molecular basis for several interfactor interactions.

One complex of central importance is the cap-binding complex eIF4F. Although the entire structure of eIF4F has not been determined, the structures of parts of the complex have been elucidated. The crystal structure of a segment of human eIF4G that binds to eIF4A has been determined, revealing a surface that interacts with eIF4A (118). The crystal structure of the ternary complex of eIF4E, an analog (7-methyl-GDP) of the 5'-cap and a peptide that corresponds to a conserved eIF4E-binding site in eIF4G, has also been determined (119). The ternary complex of eIF4E, 7-methyl-GDP, and a homologous peptide from eIF4E-binding protein (4E-BP), a regulatory protein that binds to and inhibits eIF4E's interaction with the cap, was determined in the same study. In both cases, the peptides undergo a transition from a random coil in solution to an α -helix when bound to eIF4E. While it is not yet clear whether the eIF4G peptide is structured or unstructured in the context of full-length eIF4G [although a 98-amino acid fragment of the eIF4E binding site of yeast eIF4G was shown to undergo a similar unfolded-to-folded transition upon eIF4E binding (120)], circular dichroism and NMR studies both indicate that full-length 4E-BP is not a structured protein on its own, suggesting that the unstructured-to-structured transition upon binding occurs in this context as well (119, 121, 122).

Such binding-induced unstructured-to-structured transitions may be common features of interactions between eukaryotic translation initiation factors. The interaction between eIF1A and eIF5B also involves an initially unstructured region of eIF1A (the C terminus) binding to the structured C-terminal domain of eIF5B, and becoming structured in the process (93, 123, 124). eIF1A also has a long, unstructured N-terminal tail (125) that is involved in interacting with eIFs 2 and 3 (93), and eIF1's N terminus is also long and unstructured (126), suggesting that they may undergo similar binding-induced ordering. Although it is not yet clear what these structural transitions are used for, if anything, the long, unstructured tails may be convenient to span the long distances between factors imposed by the size of the ribosome, allowing factors to communicate with one another using a minimal amount of excess protein (essentially polypeptide telephone lines). As more structures of complexes in these systems become available, it should be more obvious whether this really is a general feature of factor-factor interactions and what its purpose is.

The Molecular Mechanics of the Steps

In the following sections we discuss the current state of knowledge (and lack thereof) of the molecular mechanics underlying several key steps in eukaryotic translation initiation. Space and stamina constraints led us to choose topics of particular interest to us rather than try to survey the entire field.

TERNARY COMPLEX FORMATION AND DISSOLUTION eIF2 is also a heteromultimer, made up of three different subunits with a total mol wt of ~125 kDa. A variety of biochemical and genetic experiments have indicated that the γ subunit is the site of both GTP and Met-tRNA_i binding (6, 81, 127) and consistent with this, the γ subunit is homologous to the elongation factor eEF1A/EF-Tu (80), which is also a GTP-dependent carrier of aminoacylated tRNAs. The crystal structure of the γ subunit of archaeal eIF2 has confirmed that the protein's structure is similar to EF-Tu/eEF1A (8). Unlike eEF1A/EF-Tu, however, which must bind all elongator aminoacyl tRNAs with roughly equal affinity, eIF2 must bind only the methionylated initiator tRNA. The elements in the initiator tRNA that make this discrimination possible include three consecutive G:C base pairs in the anticodon stem, some conserved sequences in and around the T loop, a phosphoribosyl modification at position 64, and an A1:U72 base pair at the end of the acceptor stem (128). This last feature is particularly important for identifying the tRNA as an initiator tRNA (2, 129, 130). Several of the other identity elements, most notably the phosphoribosyl modification at position 64, prevent binding of the initiator tRNA to eEF1A (128, 131–133), although whether they also play nonessential roles in initiation is open to question. It was recently shown that eIF2 in its GTP-bound form has a positive contact with the methionine on the initiator tRNA and that this contact is disrupted when GTP is exchanged for GDP (e.g., upon GTP hydrolysis), whereas contacts to the body of the tRNA are not altered (2). This GTP-dependent recognition of the methionine moiety may in part prevent unacylated tRNA_i from entering the initiation pathway and is likely to be an important part of the mechanism of release of the initiator tRNA from eIF2 upon initiation codon recognition.

LOADING THE TERNARY COMPLEX ONTO THE 40S SUBUNIT A number of components of the initiation machinery seem to be involved in facilitating 43S complex formation. Early on, eIFs 1A and 3 were implicated in enhancing ternary complex binding to the 40S subunit in a reconstituted mammalian initiation system (41, 134). More recently, using the same system, it has been found that eIFs 1, 1A, and 3 are minimally required to observe stable binding of the ternary complex to the 40S subunit (43, 135, 136), whereas in a yeast-based reconstituted initiation system, only eIFs 1 and 1A are critical (137), although eIF3 does stabilize ternary complex binding in this system approximately twofold (M. Algire & J.R.L., unpublished). In most cases, it is difficult to disentangle effects on ternary complex and mRNA binding in vivo because the two are strongly

linked (38), but experiments monitoring the translationally controlled production of the transcription factor GCN4 have allowed the binding of ternary complex to be uncoupled from mRNA binding (reviewed in 90, 138). The efficiency with which the GCN4 ORF is translated is inversely proportional to the efficiency with which ternary complex binds to a 40S subunit that is already on the mRNA (because it has already translated an ORF upstream of GCN4's). Studies using this system have shown that *in vivo* both eIF1A (93) and eIF3 (42) affect ternary complex binding. In addition, *in vitro* translation experiments using yeast cell extracts indicate that eIF5 affects ternary complex recruitment and/or mRNA binding to the 40S subunit, although given the coupling between the two events, it is uncertain which step is affected. However, because eIF5 is a central component of the multifactor complex, interacting with the ternary complex in addition to eIFs 1 (111) and 3, both of which bind the 40S subunit, eIF5 is likely at least to stabilize ternary complex binding to the 40S subunit via the chelate effect.

How then do these factors facilitate 43S complex formation? Some, as suggested for eIF5, might stabilize ternary complex binding via simultaneous interactions with the ternary complex and the 40S subunit. Others could alter the conformation of the ribosome either locally or globally in such a way as to promote ternary complex binding. Small factors such as eIFs 1 and 1A might alter the local conformation of the eIF2 binding site, which is thought to be over and around the P site (139–141), whereas a large factor such as eIF3 might distort the conformation of the entire 40S subunit to allow easier access of eIF2 with its attached Met-tRNA_i. This latter possibility might explain the puzzle of why eIF3 is so big.

MOLECULAR SNAKE HANDLING: GETTING THE mRNA ON THE RIBOSOME The presumed first step in loading of the mRNA onto the small ribosomal subunit is the recognition of the mRNA's 5'-cap. The structure of eIF4E in complex with the cap analog 7-methyl-GDP has been determined by both X-ray crystallography (142, 143) and NMR (144), revealing the molecular basis for the factor's specificity for 7-methylguanosine: base stacking of the positively charged 7meG between two conserved electron-rich tryptophans and a network of other interactions. The importance of the eIF4E-cap interaction has been demonstrated both *in vivo* and *in vitro*. For example, *in vitro* translation of capped mRNAs is enhanced 3- to 30-fold relative to the uncapped message in both yeast (61, 63, 145) and mammalian (62, 145–147) extracts. Depletion of active eIF4E in the extracts abrogates the effect of the mRNA cap on translational efficiency (61), as expected if eIF4E mediates the interaction of the cap with the translational machinery. At high concentrations, uncapped mRNA is translated as efficiently as capped mRNA in extracts (147), consistent with the idea that the role of the cap is to enhance binding of the mRNA to the preinitiation complex. Electroporation of capped and uncapped mRNAs into plant, yeast, and mammalian cells

has also shown that the cap enhances translation *in vivo* 3- to 20-fold (62, 63, 148, 149), consistent with the *in vitro* results.

Careful measurements of the affinity of eIF4E for a variety of cap analogs showed a strong correlation between the analogs' affinities and their abilities to inhibit translation *in vitro* (150), suggesting that modulation of the strength of the cap-eIF4E interaction could be important for controlling rates of translation. For example, mRNAs with 5'-caps partially occluded by structures in their 5'-UTRs would be expected to have an intrinsically weaker cap-eIF4E interaction than messages with unobscured cap structures, i.e., some mRNAs are "strong" and can compete well for binding to eIF4E, whereas others are "weak" and compete poorly. However, this notion is as yet unproven.

In general, a stable secondary structure near the 5'-cap seems to be more inhibitory than is a structure farther from the 5'-end, consistent with the strong versus weak mRNA model (151–155), although several other models could also account for this effect. It is not clear that a simple hairpin structure, as used in most of the studies cited, would seriously occlude the cap. UV cross-linking studies have, in fact, suggested that the cap remains fully accessible to eIF4E even when structures are present near to it (156, 157). Based on these experiments, a model was proposed in which eIF4E can associate with the cap even when the 5'-end of the mRNA has a stable structure in it, but functional engagement requires association of one or more of the other subunits of eIF4F (or factors associated with eIF4F) with the 5'-end of the mRNA, and it is this interaction that is inhibited by secondary structures. This model was supported by a study showing that the binding of iron regulatory protein-1 (IRP-1) to its hairpin binding site in the 5'-UTR prevented stable association of eIF3 with the mRNA, but did not affect binding of the eIF4F complex (158). According to this model, the IRP-1-stabilized structure prevents eIF4G from recruiting eIF3, which in turn prevents recruitment of the 40S subunit to the mRNA. Some structures in the 5'-UTR may also prevent binding of the 40S subunit but have no effect on initiation factor binding.

The role of the eIF4F-cap interaction is proposed to be twofold. First, it brings eIF4A to the 5'-end of the mRNA where it can unwind secondary structures found there and make a suitable binding site for the 43S complex. Second, it brings factors such as eIF3 to the 5'-end of the mRNA that help the 43S complex bind to the newly ironed out mRNA (159). Data supporting the first role of eIF4F have been obtained using *in vitro* initiation systems. Initiation on small, unstructured model mRNAs does not require any of the components of the eIF4F complex, ATP or a 5'-cap, nor is it stimulated by them (9, 41, 137). Furthermore, a natural mRNA with an unstructured 5'-UTR can be recruited to a 43S complex without the need of the eIF4F complex, ATP or a 5'-cap, whereas these components are required if the 5'-UTR has significant secondary structure (21). In addition, a dominant negative mutant of eIF4A lacking *in vitro* ATPase and RNA helicase activities was less inhibitory to translation initiation on an mRNA with a low amount of secondary structure in its 5'-UTR than on an mRNA with

a very structured 5'-UTR (160). As the mutant eIF4A was incorporated into the eIF4F complex even more efficiently than the wild-type factor, these data suggest that eIF4F functions in a step slowed by increasing secondary structure in the 5'-end of the mRNA, consistent with the idea that eIF4A within the eIF4F complex unwinds these structures.

What is known about eIF4A's RNA helicase activity? eIF4A is an RNA-dependent ATPase (161) that can unwind RNA duplexes *in vitro* (50–52, 162). Its ATPase activity is required for duplex unwinding *in vitro* (162) and translation initiation *in vivo* (163), consistent with the notion that it uses energy from ATP hydrolysis to disrupt RNA structure. The enzyme undergoes a cycle of changes in its conformation and RNA affinity as it binds ATP, hydrolyzes it, and releases ADP (164, 165). These changes in RNA affinity and conformation could be used to transduce the energy from ATP hydrolysis into the breaking of RNA base pairs. The enzyme's structure is similar to that of a dumbbell, with a compact N-terminal domain connected to a compact C-terminal domain by an unstructured 11-amino acid linker (166–168). The highly conserved Walker A and B motifs involved in ATP binding and hydrolysis are found in the N-terminal domain, but other conserved residues known to be involved in ATP hydrolysis and in transducing RNA binding into ATPase activation are in the C-terminal domain (162, 167, 169), which suggests that some of the conformational changes observed involve engagement and disengagement between the two compact domains of the protein.

Unlike DNA helicases, eIF4A has no preference for unwinding duplexes with either a 5' or a 3' single-stranded overhang (50, 170, 171), and it has recently been shown that the protein can in fact unwind blunt-ended RNA duplexes with nearly the same efficiency as it unwinds duplexes with single-stranded overhangs (170). One possible interpretation of these results is that eIF4A, unlike most DNA helicases, does not translocate along the single-stranded region of the RNA as part of its unwinding mechanism; directional binding and processive translocation along single-stranded nucleic acids is thought to give DNA helicases their specificity for duplexes with 3' or 5' single-stranded overhangs (172). In support of this view, recent kinetic analyses of the RNA-dependent ATPase and RNA duplex unwinding activities of eIF4A strongly suggests that eIF4A does not function in a processive manner *in vitro* (57, 164, 171). *In vitro* at least, eIF4A is ineffective in unwinding RNA duplexes longer than ~10 base pairs and its activity drops off dramatically even for these short duplexes if the stability is increased by increasing the G:C content (170, 171). This low processivity of eIF4A might actually make physiological sense because it is unlikely that in the cell the factor would often encounter long stretches of duplex within an mRNA. Thus, unwinding short regions of secondary structure might be exactly what eIF4A has evolved to do.

Incorporation of eIF4A into the eIF4F complex stimulates its RNA-unwinding activity several fold (50, 57). eIF4B also stimulates the *in vitro* RNA-unwinding activity of eIF4A (57, 171). There is evidence that eIF4B affects the affinity of

eIF4A for its RNA (173) and ATP (174) substrates, and thus interaction of the two proteins (never directly detected) might alter the conformation of eIF4A in mechanistically important ways. A second RNA-binding protein recently discovered in mammalian systems, called eIF4H, has a similar effect on eIF4A's RNA-unwinding activity (55–57). In fact, the functions of the two factors seem to be the same: Once enough eIF4B is added to the reaction to achieve maximal stimulation of eIF4A's unwinding activity, addition of eIF4H has no additional effect, and vice versa. Similar results were found for the effects of eIF4B and eIF4H on *in vitro* translation (55). These data suggest that eIFs 4B and 4H may play functionally redundant roles in translation initiation.

Though it is unclear how eIF4A functions to unwind RNA duplexes, the general model currently (57, 174) is that eIF4A can interact with the RNA duplex in its ATP-bound form [although this interaction has not been directly detected (164)] and then upon ATP hydrolysis disrupts some of the base pairs within the helix. After a round of helix disruption, it moves along, either actively (i.e., using the energy from ATP hydrolysis) or passively (diffusion) to the next part of the helix, which it proceeds to unwind. eIF4A might simply translocate along single-stranded RNA and thus disrupt the duplexes encountered along the way, but if so, its processivity is very low. In the models, eIF4B and 4H function to capture regions of single-stranded RNA produced by eIF4A. The eIF4G subunit of eIF4F might perform a similar function via its three RNA-binding sites, which are required for efficient translation initiation (175).

Another model is related to one originally proposed by Sonenberg in 1988 (176), but that has since been largely ignored. It seems consistent with most of the current data that eIF4A might function by transiently polymerizing along single-stranded RNA and in the process disrupt RNA duplexes. The factor's ATPase activity could affect the polymer lifetime and influence the stability of the RNA duplex by modulating protein-protein and protein-RNA contacts [it is known to do the latter; (164)]. Consistent with this model, the concentration of eIF4A in yeast and mammalian cells has been estimated to be $\sim 50 \mu\text{M}$ (110, 177), comparable to that of actin (178). In *in vitro* unwinding reactions, eIF4A must always be present at high concentrations (μM) and superstoichiometric ratios relative to the RNA substrate (typically $>500:1$) in order to observe activity. Both observations are consistent with the need to drive an unstable binding equilibrium involving the factor. Also consistent with the polymerization hypothesis is the fact that when the rate of RNA unwinding is measured as a function of the concentration of eIF4A, a lag is often observed at low concentrations of the factor (51, 174; J.R.L. & D. Herschlag, unpublished), suggesting a higher-order assembly process might be taking place (i.e., the curve could be sigmoidal). This model would also explain the apparent bidirectionality of unwinding: Polymerization could take place from either end once the factor binds to the single-stranded RNA, so it could disrupt a duplex on either side. The eIF4F complex might serve as a nucleation center for the polymerization of eIF4A, which could be especially important since nucleation is often the rate-limiting

step in polymerization processes. It might also explain why a number of ATPase-deficient mutants that bind eIF4G quite well produce dominant negative phenotypes (160, 179)—polymer poisoning by mutant subunits is a well-established phenomenon. eIF4B might serve to capture the unwound RNA, as in the current model, and then hand it off to the ribosome (180).

Another possibility is that eIF4A might not be an RNA helicase *in vivo*. For example, it could function to remove proteins associated with the mRNA prior to translation. In fact, it was recently shown that inhibition of 43S-mRNA complex formation *in vitro* by the mRNA-binding protein p50/YB-1 can be partially reversed by addition of eIF4F (181). Also possible is that eIF4A acts to unwind or rearrange RNA structures, but rearranges rRNA rather than mRNA, perhaps to cause a conformational change in the 40S subunit that facilitates mRNA loading. Another RNA-dependent ATPase of the DEAD box family, Ded1p, unwinds RNA helices *in vitro* (182) and is involved in an early step of translation initiation (183, 184). Ded1p may well unwind structures in the 5'-UTR of mRNAs or perform any of the other possible functions mentioned above. Overexpression of Ded1p suppresses the effect of a mutation in eIF4E, suggesting that the factor may be involved in the mRNA recruitment step (184). Consistent with this proposal, a mutation in Ded1p is synthetically lethal with mutations in eIFs 4A, 4E and 4G. However, eIF4A and Ded1p appear not to be functionally redundant (183, 184).

The giant multisubunit factor eIF3 also may play a role in mRNA loading (38, 41, 44). eIF3 binds both the 40S ribosomal subunit and the ternary complex and several of its subunits bind RNA (185, 186). It might serve to coordinate the complicated arrangement of components required to put both the ternary complex and mRNA on the ribosome, while preventing the large ribosomal subunit from prematurely joining to the small subunit. As suggested in the previous section, eIF3 might serve as a scaffold to alter the conformation of the 40S subunit, allowing easier access for mRNA and/or ternary complex.

In addition to the events taking place at the 5'-end of the mRNA, strong evidence indicates that the 3'-end of the message also takes part in facilitating loading of the mRNA onto the 40S subunit. The key players in these 3'-end-mediated events are the 3'-poly(A) tail and the poly(A) binding protein, PAB. PAB contains four RNA recognition motifs (RRMs) and has a minimal RNA site size of ~10-nucleotides (187). Multiple molecules of PAB will bind to a poly(A) tail with approximately one PAB/27 nucleotides (188, 189). Given that the average length of poly(A) tails in yeast is ~70 nucleotides and is several fold higher in mammals, between two and ten PAB molecules will generally be bound to the end of an mRNA. The crystal structure of the two N-terminal RRM of human PAB in complex with an 11-nucleotide long poly(A) molecule was recently determined (187). The protein resembles a trough, with the two RRM forming a single, contiguous RNA-binding site. Specific recognition of the adenine bases is mediated by conserved residues in the protein via a complex assortment of hydrogen bonds, van der Waals interactions, and aromatic stack-

ing. RRM2 appears to contain the binding site for eIF4G (190) and the structure reveals a strip of highly conserved residues suggestive of a binding site on the side of this domain opposite the RNA-binding cleft. Thus the picture that arises of the poly(A) tail of an mRNA is that it is coated with PAB protomers that form a multivalent attachment site for eIF4G.

The 3'-poly(A) tail stimulates translation initiation both *in vivo* and *in vitro* (61–63, 146), just as the 5'-cap does, and this effect is mediated by PAB (61, 191). *In vivo*, the effects of the cap and poly(A) tail are generally synergistic rather than additive (62). For example, addition of a cap stimulates translation of luciferase mRNA fourfold in yeast when no poly(A) tail is present on the message, but 24-fold when the poly(A) tail is present (62). The effect of the poly(A) tail is likewise increased by the presence of the cap structure. A number of controls indicated that the stability of the mRNA was not affected by addition of the cap or poly(A) tail and thus their effects were ascribed to differences in translation rather than mRNA stability.

Experiments using yeast extracts have provided evidence that the poly(A) tail facilitates the recruitment of the mRNA to the 43S complex (61). The discovery that eIF4G and PAB bind to each other (60) led to the proposal that this interaction circularizes eukaryotic mRNAs. In support of this notion, it was shown that the eIF4E and 4G subunits of eIF4F together with PAB could circularize capped and polyadenylated nucleic acids *in vitro* (192). Based on these observations, a working model has been developed in which recognition of the poly(A) tail by PAB acts as a signal that the mRNA has not been degraded and thus is fit for translation. The interaction between PAB and eIF4F synergistically promotes binding of the 43S complex to the mRNA. This synergism could be due to something as simple as the chelate effect—one interaction pays the entropic price of reduction in degrees of freedom upon binding, allowing more benefit to be gained from the second interaction. Alternatively, or, more likely, in addition, the effect could be due to conformational changes in PAB and eIF4F induced by binding to one another that have positive effects on the steps facilitated by these factors. There is evidence to suggest that binding of PAB to eIF4F affects eIF4F's cap-binding and ATPase activities (115, 193–195). PAB and eIF4F could also affect the kinetics of independent steps in the pathway, leading to synergism that does not depend on cooperative binding or mutually induced conformational changes (196).

Further support for the proposal that the poly(A) tail is involved in recruiting the 43S complex to the mRNA was derived from the observation that even though initiation on polyadenylated mRNAs lacking a 5'-cap is more efficient than on their nonpolyadenylated counterparts, these initiation events frequently take place at internal AUG codons, which is not true when the mRNAs are capped (63). The interpretation of these data was that the poly(A) tail can facilitate binding of the 43S complex to the mRNA, but it cannot efficiently direct it to the 5'-end in the absence of the eIF4F-5'-cap complex. This result provides compelling evidence that the 5'-cap helps enforce the use of only the

very 5'-end of the mRNA as the binding site for the 40S subunit. To observe similar synergistic stimulation of initiation between the cap and poly(A) tail in cell extracts as was observed *in vivo*, it was necessary either to increase the concentration of competitor RNA in the extracts (63) or to decrease the concentrations of ribosomes and associated factors (146). In addition, poly(A)⁻ mRNAs can be efficiently translated *in vivo* as long as the ribosome:mRNA concentration ratio is high (197). These results could be interpreted as suggesting a connection between the poly(A)-tail and binding of mRNA to the ribosome because either addition of competitor RNA or a decrease in ribosome concentration might be expected to adversely affect the binding of the mRNA and the 40S subunit, in the first case by competition for binding and in the second by mass action. The data are not, however, inconsistent with other possible roles for the poly(A) tail.

Whereas much of the data has suggested that the main role of the poly(A) tail and PAB is to cooperate with eIF4F and the 5'-cap to facilitate loading of the 43S complex onto the 5'-end of the mRNA, other data suggest that the poly(A) tail also affects different steps in the pathway. First, experiments in mammalian cell extracts suggested that the poly(A) tail might stimulate the subunit joining step of initiation (198, 199) rather than mRNA binding to the 43S complex. Mutations that lower the concentration of 60S subunits *in vivo* specifically decrease the efficiency with which poly(A)⁻ mRNAs are translated, a result hard to reconcile with the proposal that the main role of the poly(A) tail is to promote mRNA recruitment to the 43S complex (197). Decreasing the concentration of 40S subunits also adversely affected translation of poly(A)⁻ mRNA relative to poly(A)⁺ mRNA, however, consistent with a role for the poly(A) tail in 43S binding as well. Additionally, when yeast bearing a temperature-sensitive allele of PAB are shifted to the nonpermissive temperature, they accumulate free 60S subunits (200), as one might expect if PAB is involved in the subunit joining step. It has also been suggested that PAB may interact with the 60S subunit directly (200, 201). Thus there is now considerable evidence that the poly(A) tail and PAB may influence subunit joining in addition to its effects on mRNA binding to the 43S subunit.

The subunit joining story is not without its complications either. For example, while lowering the concentration of 60S subunits *in vivo* suppressed translation of poly(A)⁻ mRNA relative to poly(A)⁺ mRNA (197), it also obviated the need for PAB itself (200), a seemingly contradictory result if PAB and the poly(A) tail conspire to facilitate subunit joining. Furthermore, deletion of two nonessential putative RNA helicases, Ski2p and Slh1p, dramatically enhances translation of poly(A)⁻ mRNA, bringing it to levels comparable to translation of poly(A)⁺ mRNA (202, 203). Thus it has been suggested that these proteins are enforcers of discrimination against the translation of mRNAs lacking poly(A) tails. This may be a mechanism for preventing translation of nonpolyadenylated viral mRNAs (203), but it may also play a role in mediating poly(A) effects on native mRNAs. Finally, the poly(A) tail and PAB (as well as the Ski genes) have roles in mRNA degradation and stability and, while in most of the experiments

discussed above care was taken to control for effects on mRNA degradation rates, some of the effects observed might still be due to alteration of mRNA stability rather than the rates of steps in the initiation pathway.

Even the appealing closed loop model of eukaryotic mRNAs has recently been questioned. Experiments in mammalian cell extracts have demonstrated that addition of exogenous poly(A) [but not poly(dA) or poly(U)] can stimulate translation of both poly(A)⁺ and poly(A)⁻ capped mRNA (194). The stimulation was inhibited by adding a viral protein that is able to disrupt the eIF4G-PAB interaction, suggesting that the (poly(A)-PAB)-eIF4G interaction itself and not the circularization of the mRNA is responsible for the enhancement of translation initiation provided by the poly(A) tail and the cap. Adding PAB to yeast extracts stimulates translation of capped mRNAs lacking poly(A) tails (204), either via the *trans*-stimulatory effects just described or because PAB can perform functions without being bound to poly(A). Disruption of the PAB-eIF4G interaction did not prevent this poly(A) tail-independent effect of PAB, however, suggesting that it may be distinct from stimulation by exogenous poly(A). Finally, a homologue of the central region of eIF4G, called PABP-interacting protein-1 (PAIP1), that binds eIF4A and PAB but not eIF4E, has been discovered in mammals (205). Overexpression of PAIP1 stimulates translation, suggesting that this protein can enhance initiation of polyadenylated mRNAs without joining the 5'- and 3'-ends.

IN SEARCH OF THE INITIATION CODON: SCANNING AND AUG RECOGNITION The scanning model proposes that once a 43S complex is loaded onto the 5'-end of an mRNA it moves along the message toward the 3'-end looking for the initiation codon (46). Placing stable secondary structures in the middle of the 5'-UTR in the path of the ribosome can lower the rate of 80S complex assembly without diminishing the efficiency with which the 43S complex binds to the 5'-end of the message, consistent with the predictions of the model (206, 207). Toe-printing experiments in a reconstituted mammalian initiation system have shown that the 43S complex can assemble on the 5'-end of the mRNA (albeit in a nonfunctional and unstable form) in the absence of eIFs 1 and 1A, but that it will not reach the initiation codon unless the two factors are included (208). These data indicate that eIFs 1 and 1A are required for the formation of a 43S complex capable of scanning the mRNA and further support the notion that binding of the 43S complex to the mRNA and location of the initiation codon can be decoupled.

It has also been reported that the scanning process requires the hydrolysis of ATP (209), although it is not clear what this energy is used for. One possibility is that a molecular motor (e.g., eIF4A or Ded1p) uses the energy derived from ATP hydrolysis to actively translocate the ribosome in the 5' to 3' direction. The actual movement of the ribosome may occur in a passive manner via diffusion, and the ATP requirement may reflect the need for unwinding of structures in the mRNA, possibly by the action of eIF4A and/or Ded1p, in order to allow diffusive movement. Also possible is a Brownian ratchet mechanism in which an ATP-

dependent RNA unwindase located on the front (3') side of the 40S subunit unwinds structures in the mRNA, allowing the ribosome to slide past via diffusion. When the ribosome randomly slides in the 3' direction over the unwound structures, they reform behind it, preventing backsliding. In support of the diffusion-based models, it was recently shown in a reconstituted mammalian system that 43S complexes can reach the AUG codon in an mRNA in the absence of ATP and eIF4A if the 5'-UTR is unstructured but not if it contains significant secondary structure (21). These data indicate that the search for the AUG codon does not inherently require the hydrolysis of ATP and suggest that energy input is required to get through structured regions in the message. It is not yet clear, however, if the rate at which the AUG codon in an unstructured mRNA is located is affected by ATP hydrolysis or by the addition of factors not present in these experiments (e.g., Ded1p or as-yet undiscovered factors).

The molecular events that take place when the scanning 43S complex reaches an AUG codon are also unclear. Aside from the initiator tRNA, which seems to be the key point of contact with the mRNA in the decoding process (210), three players appear to be central to initiation codon location: eIF1, eIF2, and eIF5. Each has been found in genetic screens to affect the fidelity of initiation codon selection (31, 32, 34, 211–214), allowing initiation at a UUG codon (but oddly in most cases, not at other near-cognate codons). The mutations in eIF1 that affect the fidelity of initiation site selection all cluster at the top of a strip on the surface of the molecule that is made up of highly conserved residues and is suggestive of the binding site for another component of the system (126). A role for eIF1 in initiation codon selection has also been demonstrated *in vitro* (21).

The mechanism of action employed by eIF1 during initiation codon recognition is not known. It may directly interrogate the anticodon-mRNA pairings and when three perfect base pairs are formed, interact with the resulting duplex and reduce the energy of the complex enough to stop scanning. It might also then send a signal to eIF2 and/or eIF5, initiating GTP hydrolysis. In this model, eIF1 might play a similar role to the decoding bases A1492, A1493, and G530 in the bacterial 30S ribosomal subunit that swing out to interact with the codon-anticodon duplex in the A site during tRNA selection in elongation, thus (presumably) signaling to EF-Tu that the cognate tRNA has been found (215). For most of the time in translation, all decoding happens in the A site and thus it may require an accessory factor (eIF1) in the P site to allow it to perform this function during initiation. The proposal that eIF1 binds in or near the ribosomal P site comes mainly from the experiments showing that bacterial IF3, a possible homologue of eIF1, binds in the P site, although IF3 does not appear to be close enough to the anticodon end of the tRNA to monitor its base-pairing interactions (216). [Note: The binding site of eIF1 was recently determined by footprinting and found to be similar to that of IF3, as predicted (216a).] A related model is that eIF1 alters the conformation of the 40S subunit's P site in such a way as to allow decoding to take place (21). eIF1 might also detect the formation of the codon-anticodon interaction indirectly, for example by interacting with the body

of the initiator tRNA and responding to conformational changes in it when the initiation codon is reached. Such conformational changes in the tRNA have been proposed to be important for sensing the cognate codon-anticodon base pairing during A-site decoding (217, 218).

The function of eIF2 in the decoding process is to respond to the discovery of the initiation codon by hydrolyzing GTP and then, at the appropriate time, release the Met-tRNA_i into the P site. Mutations that reduce initiation site selection fidelity have been isolated in all three subunits of eIF2 (34, 211, 212, 214). The initiation codon fidelity mutation in the γ -subunit is in the GTP-binding site and appears to increase both the eIF5-independent GTPase activity of eIF2 and the rate of dissociation of Met-tRNA_i from ternary complex (34). One of the mutations in the β -subunit was found to cause similar effects. Based on these data, it was proposed that these mutations allow premature release of the initiator tRNA into the P site of the 40S subunit in response to an anticodon-UUG mismatch, although the fact that they still confer a preference for UUG over other near-cognate codons suggests that it is not as simple as spontaneous GTP hydrolysis and tRNA dissociation. Instead, the barrier to these events has more likely been lowered such that UUG can now trigger them, but for whatever reasons, other near-AUG codons do not do so as efficiently. Mutations that weaken the interface between the β - and γ -subunits of eIF2 also lead to a decrease in the fidelity of initiation codon selection, and these mutations are synthetically lethal with the mutation mentioned above in the β -subunit that appears to increase the intrinsic GTPase activity of the factor (214). Thus there appears to be a complex interplay between the tertiary and quaternary structure of the factor, its Met-tRNA_i binding and GTPase activities, and its ability to respond to the location of the correct initiation codon.

eIF5 stimulates the GTPase activity of eIF2 (219). eIF5 can bind directly to the ternary complex via the β -subunit of eIF2, and this interaction is critical for eIF5's function (97, 220). In addition, eIF5 interacts more strongly in solution with eIF2·GDP·AlF₄⁻, a putative transition-state analogue of the GTP hydrolysis reaction, than with eIF2·GDP (221), consistent with its proposed role as a GAP. However, constitutively activating eIF2 would serve no useful purpose, and thus the activity of eIF5 must be regulated in response to a signal that the AUG codon has been found. This signal could be direct, e.g., eIF5 could monitor the codon-anticodon pairings itself, or it could be indirect, mediated through conformational changes in eIF1 or the 40S subunit. As with two of the eIF2 mutations described above, the mutation in eIF5 that reduces the fidelity of initiation site selection *in vivo* appears to result in hyperstimulation of eIF2's GTPase activity (34). However, *in vivo* this mutant allele produces a strong (≥ 100 -fold) preference for initiation at UUG versus other one-base changes from AUG (AUU, GUG, CUG) and thus its effect cannot be as simple as unregulated overstimulation of eIF2's GTPase activity. Instead, the barrier to its being triggered to activate eIF2 for GTP hydrolysis has probably been lowered in such a way as to allow it to be activated by the anticodon-UUG mismatch in addition

to the anticodon-AUG match. Again, the fact that it does not respond to the other near-AUG codons is probably significant.

While eIFs 1, 2, and 5 appear to be the core of the AUG-recognition apparatus, at least as far as initiation factors are concerned, evidence is mounting that other factors may have a hand in the process as well. For example, a mutation in eIF4G that weakens its interaction with eIF1 produces a modest initiation site selection fidelity phenotype (111). Remarkably, this mutation, like those in eIFs 1, 2, and 5 described above, increases initiation at a UUG codon but not at AUU or UUA codons. Overexpression of eIF1 could suppress the phenotype, consistent with the interpretation that the effect results from a decrease in the binding of eIF1 to eIF4G. One of the initiation codon-selection fidelity mutants in eIF1 weakened binding of the factor to eIF4G, further suggesting that this interaction is important in initiation codon location (111). One possible interpretation of these data is that eIF4G remains bound to the 43S complex as it scans the mRNA in search of the initiation codon and that it helps to organize the complex to respond appropriately to the AUG's identification. Because eIF3 interacts with eIFs 1, 2, and 5, it has been proposed that it too may play a role in locating the initiation codon (35, 92).

A wide variety of experiments have established that in mammals the sequences surrounding AUG codons play a role in specifying which one is used as the initiation site (222). The first AUG in the mRNA can be bypassed if it is in an unfavorable sequence context and a downstream AUG imbedded in a favorable context will be used instead. The mechanism through which this Kozak sequence exerts its effect remains mysterious, however. Attempts to find sequences in the 18S rRNA that interact with these regions, analogous to the role of the anti-Shine-Dalgarno in 16S rRNA, have thus far proved futile (45). Recent *in vitro* experiments, however, have suggested that eIF1 might play a role in discriminating between AUGs in favorable and unfavorable contexts by destabilizing preinitiation complexes on "incorrect" AUGs (21). Thus something about the sequence around an AUG codon may allow it to either engage or not engage correctly with the apparatus that is searching for the initiation codon in the P site, although how is unclear. Also possible is that some part of the preinitiation complex (e.g., eIF1) directly monitors the sequences around the AUG codon.

By contrast, yeast and plants have very different sequences around their initiation sites [AAAAAUGUCU and AA(A/C)AAUGGC, respectively; (223–225)] than do mammals (GCCACCAUGG), the only common nucleotide being the A at position -3 (with the A of AUG as 0). A variety of experiments have shown that in yeast the consensus sequence has only a small effect on the efficiency of use of an AUG codon as the initiation site (154, 226–229), in contrast to the larger effects usually seen in mammalian systems. This apparent lack of an important role for the consensus sequence in yeast mRNAs may be message dependent, as there has been at least one report in which it influences the choice of initiation codons by up to tenfold (230). Also unclear is whether the

context of the AUG codon plays a significant role on initiation site selection in plants (225). Given the differences among the sequences surrounding the initiation codons in these diverse eukaryotes, the mechanism of action of these nucleotides in mammals may not be general to all eukaryotes. Finally, even the situation in mammals appears to be more complicated than originally thought. An analysis of genes from the human genome has indicated that as many as 40% of mRNAs have AUG codons upstream of the initiation site and that the consensus sequence around the initiation codon may not be as strongly conserved as originally proposed (231). Based on these data, it has been suggested that initiation of translation on many mRNAs occurs via nonstandard pathways, for example internal ribosome entry or leaky scanning past upstream AUGs (231), a controversial conclusion, however, in part because the analysis requires that the initiation site be known for each mRNA, which is not always a straightforward matter (232).

BRINGING THINGS TOGETHER: SUBUNIT JOINING The final (known) step of eukaryotic translation initiation is the joining of the 40S and 60S subunits following initiation codon recognition and deposition of the initiator tRNA into the P site of the 40S subunit. Considerable progress has been made in the past few years concerning the mechanics of this step. It was long thought that only a single GTP hydrolysis event was required for eukaryotic translation initiation (233). However, the discovery that a yeast homologue of bacterial IF2, eIF5B, was a GTPase and a central initiation factor (17) and a kinetic dissection of the initiation process in an *in vitro* mammalian system (9) both indicated that two GTP hydrolysis events are required, one catalyzed by eIF2 upon initiation codon recognition and another at the end of the pathway, after 80S complex formation (9). This requirement was subsequently confirmed both *in vivo* and *in vitro* (18, 29, 234). eIF5B, originally called eIF5 (41, 235) before being rediscovered as a separate initiation factor, was subsequently shown to facilitate subunit joining (30). eIF5B's GTPase activity is not actually required to bring the subunits together. Instead, GTP hydrolysis promotes the release of the factor from the 80S complex once the subunit joining step has been completed (29, 30, 234).

The crystal structure of an archaeal eIF5B has been determined without nucleotide and bound to GDP and GDPNP (28). These structures reveal an overall shape resembling a chalice, with three domains on top, including the GTPase domain, connected to a fourth domain on the bottom via a long α -helix. The top and bottom of the chalice undergo small movements relative to each other when GDPNP is replaced by GDP, suggesting that GTP hydrolysis is used to induce conformational changes in the factor. These changes could potentially be employed for mechanical work on the 80S complex. For example, eIF5B might move the initiator tRNA into its final position in the P site using the energy from GTP hydrolysis (8), similar to proposals for the role of EF-G/EF2 during translation elongation (236). However, the idea that the essential role of eIF5B's GTP hydrolysis activity was to reorganize the initiation complex was cast into

doubt when it was found that the key translational functions of a GTPase-deficient mutant of eIF5B could be restored by a second mutation that reduced the affinity of the factor for the ribosome without restoring its GTPase activity (29). These and other data have indicated that the GTPase activity serves as an on/off switch: In its GTP-bound form, eIF5B has a high affinity for the ribosome and in its GDP-bound form, a low affinity. If the affinity is decreased by a mutation such that it can dissociate from the ribosome at a reasonable rate in its GTP-bound form, the factor can still perform all of its essential functions even without being able to hydrolyze GTP. The main role of the conformational change in eIF5B, then, appears to be to change the affinity of the factor for the ribosome. This could easily be achieved by the relatively small changes observed, either by creating a steric clash between the factor and the ribosome or by breaking important positive interactions.

Experiments testing the effects of the GTPase-deficient mutants of eIF5B on the translation of the GCN4 mRNA (138) indicated that while eIF5B's GTPase activity is not required for the core process of translation or for maintaining reasonably healthy yeast cells, its disruption increases the frequency with which ribosomes fail to begin translation at upstream initiation codons and proceed downstream (29). This suggests that the timing of GTP hydrolysis plays a regulatory function and that premature release of the second-site-suppressed eIF5B mutant from the 80S complex leads either to movement of the complex past the AUG where it was located or to premature dissociation of the Met-tRNA_i followed by downstream scanning of the message and reinitiation. This model predicts that GTP hydrolysis by eIF5B is not triggered until the factor somehow senses that the complex is correctly set up to begin elongation. This signal could be that additional factors have been released or that the initiator tRNA is in the right place within the complex. An alternative model that still cannot be ruled out is that GTP hydrolysis by eIF5B, while not being required in most instances to mechanically reorganize the initiation complex, can jiggle into place incorrectly set-up complexes that occur occasionally during normal initiation. The failure by the second-site-suppressed GTPase-deficient mutant to jiggle these aberrant complexes might lead to the missed-AUG effects observed.

Despite recent advances in understanding the subunit joining step of eukaryotic translation initiation, the molecular mechanisms used by eIF5B to facilitate the process remain to be elucidated. First, it is not yet clear whether eIF5B binds to the 40S subunit before the 60S subunit or vice versa. The exact timing of GTP hydrolysis by eIF2 and eIF5B relative to binding of eIF5B to the ribosomal subunits and subunit joining have also not yet been fully explored. How does eIF5B actually facilitate the joining of the subunits? Binding of eIF5B to the 40S and/or 60S subunit could well alter the subunit's conformation in such a way as to increase the rate constant for the joining reaction. Also possible is that binding of eIF5B to one subunit presents a new molecular surface that facilitates the docking of the other subunit. What actually triggers eIF5B's GTPase activity? eIF5B is suggested to facilitate the release of other factors, such as eIF1A, from

either the 40S subunit or final 80S complex (93, 123), although if so and how remain to be explored. Finally, as discussed above, understanding how eIF5B checks or otherwise ensures that the final 80S complex is properly set up to elongate a polypeptide may provide important insights into how the translational machinery maintains quality control in protein synthesis.

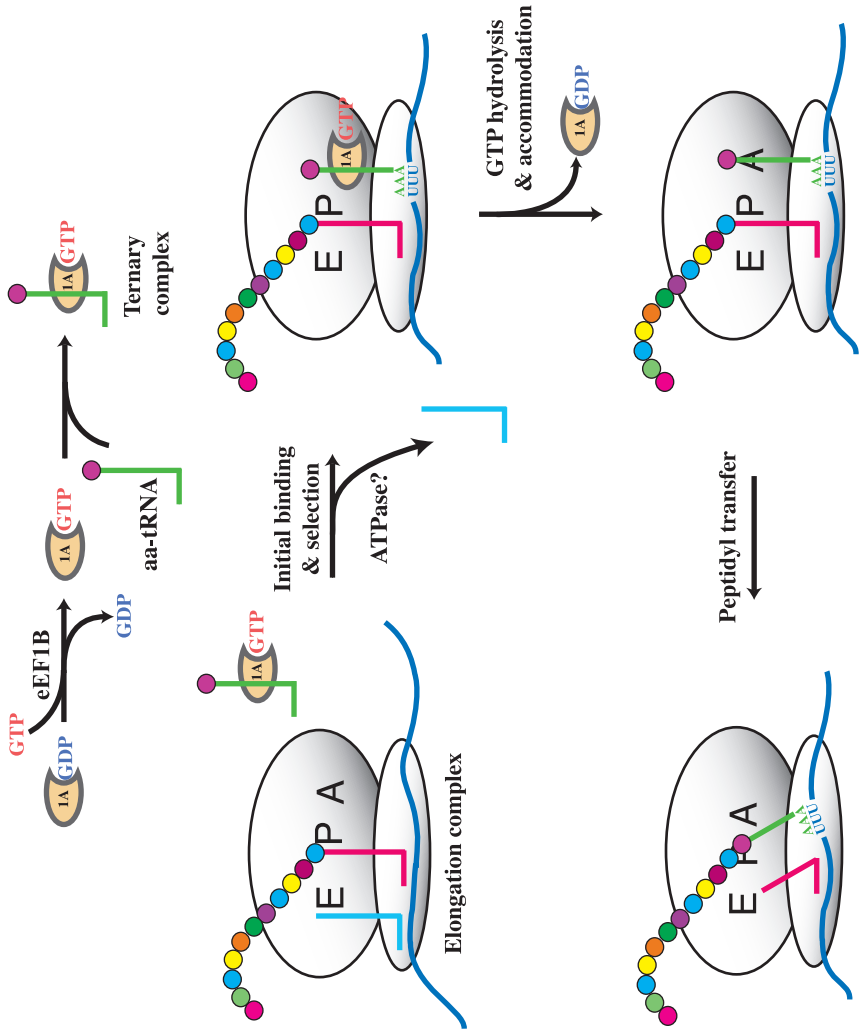
ELONGATION

In contrast to the initiation and termination stages of translation, the machinery used during the course of translation elongation has been highly conserved across the three kingdoms of life. Because of this conservation it is assumed that the mechanisms underlying elongation are the same in eukaryotes as they are in bacteria and archaea (reviewed in 237, 238). We therefore outline only briefly the current state of the field and then focus on one intriguing aspect that appears to be unique to one branch of the eukaryotes.

Peptide chain elongation begins with a peptidyl tRNA in the ribosomal P site next to a vacant A site (Figure 3). An aminoacyl tRNA is carried to the A site as part of a ternary complex with GTP and the elongation factor 1A (eEF1A; EF-Tu in bacteria). eEF1A·GTP·aa-tRNA ternary complexes with either the cognate or noncognate aminoacyl tRNAs can bind to the ribosomal A site. However, several steps involving codon-anticodon base pairing between the mRNA and the tRNA, conformational changes in the decoding center of the small ribosomal subunit, and GTP hydrolysis by eEF1A/EF-Tu ensure that only the cognate tRNA is selected for entry into the next stage of elongation (reviewed in 239). Codon-anticodon base pairing induces three bases in the small ribosomal subunit's rRNA to swing out and interact with the resulting mRNA-tRNA duplex (215). This in turn appears to activate eEF1A/EF-Tu's GTPase activity. eEF1A·GDP releases the aminoacyl tRNA into the A site in a form that can continue with peptide bond formation.

The ribosomal peptidyl transferase center then catalyzes the formation of a peptide bond between the incoming amino acid and the peptidyl tRNA (240). The result is a deacylated tRNA in a hybrid state with its acceptor end in the exit (E) site of the large ribosomal subunit and its anticodon end in the P site of the small subunit (241). The peptidyl-tRNA is in a similar hybrid situation with its acceptor end in the P site of the large subunit and its anticodon end in the A site of the small subunit. This complex must be translocated such that the deacylated tRNA is completely in the E site, the peptidyl tRNA completely in the P site, and the mRNA moved by three nucleotides to place the next codon of the mRNA into the A site. This task is accomplished by elongation factor 2 (EF-G in bacteria), which hydrolyzes GTP as it facilitates translocation (242). This cycle is repeated until a stop codon is encountered and the process of termination is initiated.

Following the hydrolysis of GTP and the release of aminoacyl tRNA onto the ribosome, eEF1A·GDP is released and must be recycled to its GTP-bound form



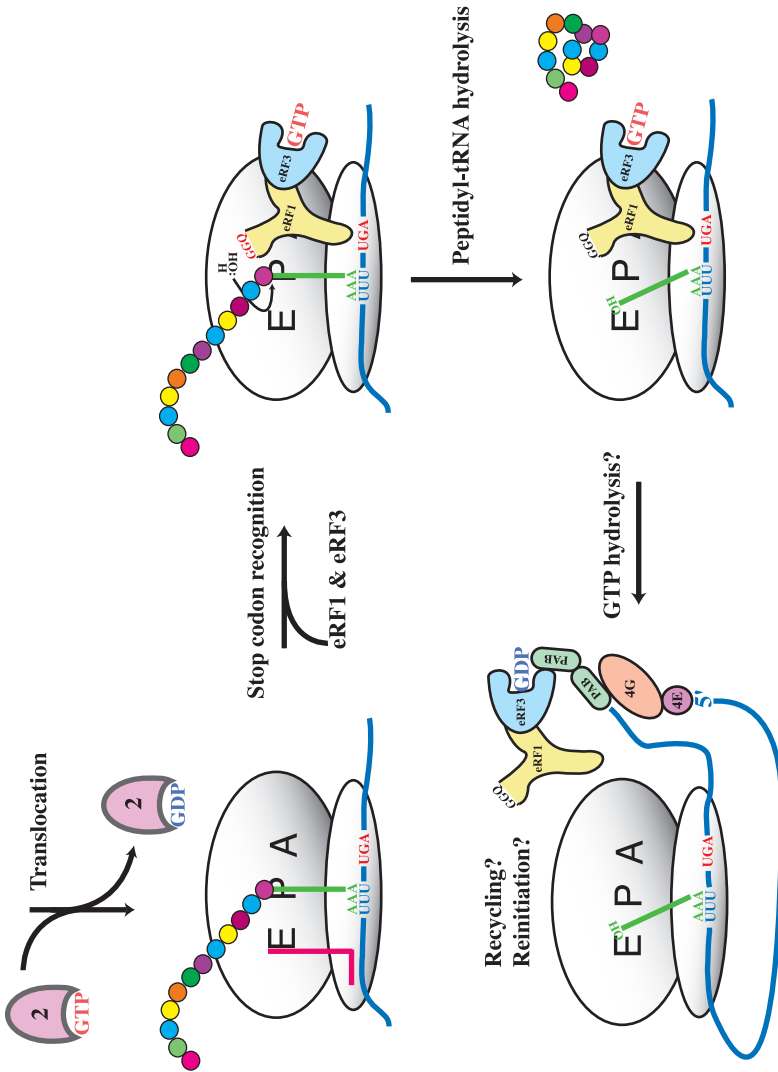


Figure 3 Cartoon depicting the current models for translation elongation and termination in eukaryotes. Not all steps are shown and several steps have been condensed. It has also been assumed that the molecular mechanics of elongation are conserved between bacteria and eukaryotes. The lines between the UUU and UGA codons are not meant to represent mRNA, but are merely for clarity.

so that it may participate in successive rounds of polypeptide elongation. A multifactor complex called eEF1B (eEF1B α plus eEF1B β , formerly called eEF1B γ) catalyzes this exchange. Currently, no known guanine-nucleotide exchange factor (GEF) exists for either EF-G or eEF2.

Human eEF1A and *E. coli* EF-Tu are 33% identical overall, with even greater identity between their GTP-binding domains (243). The archaeal EF1A from *Halobacterium halobium* is 49% identical to yeast eEF1A and 36% identical to *E. coli* EF-Tu (244). Among EF2 homologs, aEF2 from *H. halobium* is 36% identical to eEF2 from *Saccharomyces cerevisiae* and *Drosophila melanogaster* and 30% identical to *E. coli* EF-G (245). Aside from the extensive sequence similarity, X-ray crystallographic studies and cryo-EM reconstructions have demonstrated remarkable structural similarity between EF1A and EF2 homologs on and off the ribosome (246–249). One interesting exception to these similarities is a variety of posttranslational modifications of both factors in eukaryotes (250), including the conversion of a histidine residue in eEF2 to a diphthamide (251), which is the target of ADP-ribosylation by diphtheria toxin.

Perhaps the most significant exception to the rule of evolutionary conservation in translation elongation is the existence of elongation factor 3 (eEF3) exclusively in fungi. eEF3 was shown to be required when yeast ribosomes are used in poly-Phe synthesis assays in vitro employing yeast eEF1A and eEF2 but not when mammalian ribosomes are used in the same system (252). Furthermore, the gene encoding eEF3 has been shown to be essential for viability in yeast (253). eEF3 possesses ribosome-dependent ATPase and GTPase activities (254) and contains a duplicated nucleotide binding motif homologous to those found in the typically membrane-associated ATP Binding Cassette (ABC) proteins (253), such as the cystic fibrosis transmembrane conductance regulator. eEF3 is found primarily associated with translating cytosolic ribosomes, mostly in polysome fractions, and is required for each round of peptide bond formation. It interacts with eEF1A (255) and this interaction is important for protein synthesis in vivo (256). eEF3 is thought to facilitate release of the E site-deacylated tRNA and to enable efficient binding of the eEF1A·GTP·aa-tRNA ternary complex to the A site (257). The data also suggest that binding of tRNA to the A and E sites is anticoupled. ATP hydrolysis by eEF3 appears to be required for its effects.

Why do fungal ribosomes require a distinct elongation factor to facilitate E site clearance and A site loading when other eukaryotic, bacterial, and archaeal ribosomes apparently do not? It has been suggested that mammalian ribosomes actually possess an intrinsic eEF3-like activity. Very stable ATPase and GTPase activities, lost only after exposure to 4 M LiCl, are associated with mammalian ribosomes (258). ATP hydrolysis has also been reported to stimulate release of E-site-bound tRNA from mammalian ribosomes (259), and occupancy of the A site appears to stimulate the intrinsic ATPase activity (258). However, no candidate in mammalian ribosomes has yet been identified as the homologue of fungal eEF3. *E. coli* possesses a soluble ribosome-dependent ATPase, RbbA, that stimulates poly-Phe synthesis in vitro, requires the hydrolysis of ATP for its

effects, and has some sequence similarity to eEF3 (260, 261). Thus it remains possible that bacteria may have an eEF3-like activity as well.

TERMINATION

The termination of translation (reviewed in 262–267) occurs in response to the presence of a stop codon in the ribosomal A site. The end result of this process is the release of the completed polypeptide following the hydrolysis of the ester bond linking the polypeptide chain to the P site tRNA. The peptidyl transferase center of the ribosome is believed to catalyze the hydrolysis reaction (268–273), in response to the activity of class 1 release factors, which decode stop codons presented in the A site. Class 2 release factors are GTPases that stimulate the activity of class 1 release factors regardless of which stop codon the class 1 factor has engaged (Figure 3).

Comparative Termination

BACTERIA Bacteria possess two class 1 release factors, RF1 and RF2, with overlapping codon specificity, such that each responds to UAA, whereas UAG is decoded only by RF1 and UGA is decoded only by RF2 (274). The swapping of RF1- and RF2-specific domains among RF1/RF2 chimeric factors led to the identification of a putative peptide “anticodon” within each factor; the sequences PAT in RF1 and SPF in RF2 were capable of imposing either RF1 or RF2 specificity on the chimeric factor (275). Thus, it was concluded that the prokaryotic factors employ a localized linear amino acid sequence for the recognition of a stop codon. The peptide anticodon and the stop codon are thought to form an interaction that is analogous to the codon:anticodon pairing between mRNA and tRNA. These data have greatly influenced the study of eukaryotic translation termination, as discussed below.

However, swapping the discriminatory domain between native factors, consisting exclusively of RF1 or RF2 domains, as opposed to a particular mixture of RF1 and RF2 domains, resulted in an inactive release factor. This suggests that the codon:anticodon base-pairing analogy is an oversimplification of the actual mechanism of stop codon recognition by RFs 1 and 2. Structures present in other domains most likely help define the codon recognition ability of each factor, perhaps by influencing the structure of the discriminator region.

In bacteria RF3 is the class 2 release factor, which not only stimulates the activities of RF1 and RF2, but is also required to eject them from the ribosome following peptidyl-tRNA hydrolysis (276–278).

EUKARYOTES In contrast to the prokaryotic factors, only one class 1 release factor is present in eukaryotes. Accordingly, eRF1 has an omnipotent decoding capacity and can promote the hydrolysis of peptidyl-tRNA in response to any of

the three stop codons, UAA, UAG, or UGA (279–281). In organisms that utilize variant genetic codes, such as the ciliates, in which either UGA or both UAA and UAG have been reassigned as sense codons, eRF1 decoding potential is appropriately restricted by mechanisms that are currently not well understood.

Eukaryotes also possess a single class 2 release factor, eRF3 (282, 283). In contrast to RF3, a role for eRF3 in triggering the release of eRF1 from the ribosome following peptidyl-tRNA hydrolysis has yet to be experimentally verified. Furthermore, eRF3 is an essential protein in eukaryotes, whereas RF3 is dispensable for viability in bacteria (276, 277, 284). eRF1 and eRF3 bind to each other in the absence of the ribosome, and this interaction is required for optimum efficiency of termination in *S. cerevisiae*. In contrast, no such cytosolic complex between RFs 1 or 2 and RF3 has been observed (285–288), although transient interactions on the ribosome between the class 1 factors and RF3 have been proposed (273).

ARCHAEA Translation termination, as far as stop codon recognition and peptidyl-tRNA hydrolysis are concerned, appears to be similar between archaea and eukaryotes based on the homology of aRF1 and eRF1 as well as the discovery that *Methanococcus jannaschii* aRF1 is fully functional in an in vitro release factor (RF) assay employing mammalian ribosomes (281). Surprisingly, no gene encoding an archaeal homologue of eRF3 has been identified, although this does not necessarily mean that there is no archaeal counterpart to eRF3. It has been suggested, however, that a process of reductive evolution of the translational apparatus occurred during the divergence of archaea (83), which may have made such a factor unnecessary.

IS THE MECHANISM OF TERMINATION CONSERVED? The lack of sequence homology between bacterial and eukaryotic factors might indicate independent origins of the process of termination (280, 282). Alternatively, these factors may have diverged so much that their lack of resemblance belies a true evolutionary relationship. In fact, there appears to be one universally conserved sequence motif among class 1 release factors, the GGQ motif, which is required for the activation of peptidyl-tRNA hydrolysis (272, 289). The universal conservation of the core ribosomal proteins and rRNA (83, 237) also suggests that the molecular mechanism of the peptidyl-tRNA hydrolysis reaction, which is catalyzed by the ribosome, is likely conserved. However, as there are no other apparent regions of similarity aside from the GGQ motif in class 1 release factors from bacteria and eukaryotes/archaea, the mechanism of stop codon decoding may not be conserved. Furthermore, the fact that there are two class 1 factors in bacteria but only one in eukaryotes and archaea also suggests important differences in decoding mechanisms. One similarity, however, is that the termination signal in both bacteria and eukaryotes consists not of a three-base codon, but includes the +4 position as well (290–293), suggesting vestiges of a primordial termination

codon-recognition mechanism. Finally, there are no homologous sequences between RF3 and eRF3 aside from their GTP-binding motifs (282).

The Structure of eRF1

The three-dimensional structure of eRF1 has been determined (294). The overall shape of the protein resembles the letter “Y,” each arm being formed by a distinct domain. Domains 2 and 3 form the branches of the Y and domain 1 forms its base. Together, domains 1 and 2 are thought to constitute the core of eRF1 responsible for ribosome binding, stop codon recognition, and the activation of peptidyl-tRNA hydrolysis (295). Domain 3 is required for the binding of eRF3 (296, 297), while all three domains are required to activate eRF3's GTPase activity, as it is the ribosome-bound eRF1 that functions as the GAP (283).

Interestingly, the crystal structure reported for *E. coli* RF2 differs significantly from that of eRF1, raising doubts, as mentioned above, as to the evolutionary conservation of stop codon decoding (294, 298). In this structure, the GGQ motif and the putative SPF tripeptide anticodon were only 23Å apart, much too short to bridge the gap between the peptidyl transferase center and the mRNA. However, a more recent cryo-EM structure revealed that RF2 undergoes significant reorganization when it binds to the ribosome, such that the helix contiguous with the GGQ loop rotates outward, thereby increasing the distance to nearly 60Å (299, 300). An extended conformation on the ribosome was also indicated by hydroxyl radical probing studies (301). As it now appears that RF2 on the ribosome adopts a structure similar to that of eRF1, these factors may be evolutionarily related after all. Alternatively, this could be an example of convergent evolution dictated by the constraints imposed by the ribosomal A site.

Domain 1 of eRF1: The Mechanism of Stop Codon Decoding

Several mutations in eRF1 were isolated by a genetic screen for a unipotent suppressor phenotype; the suppression of only one of the three stop codons rather than a general stop codon recognition defect (302). These mutations are localized near the interface between two α -helices and an opposed β sheet in domain 1. The van der Waals surface of this interface constitutes a continuous groove hypothesized to be the site of mRNA interaction. Because all mutant eRF1s isolated by the screen also displayed weak omnipotent suppression phenotypes, it was suggested that the decoding of stop codons in eukaryotes is a holistic process; that is, recognition of one stop codon cannot be completely decoupled from recognition of the others. This “articulated coupling” hypothesis advocates that the decoding of stop codons in eukaryotes is more complicated than, and might not be directly analogous to, the decoding of sense codons by tRNA (302).

Additional data concerning the molecular basis for stop codon discrimination have been obtained from experiments involving eRF1 proteins from variant genetic code organisms that have reassigned either UAG or both UAA and UAG

to function as sense codons (reviewed in 303). For these changes to be effective, the reassigned codons must no longer be recognized by eRF1 as signals for the termination of translation. It has been proposed that the residues that determine the specificity of eRF1 for particular stop codons can be identified using the sequence divergence of variant and universal code eRF1s (304). Based on these sequence comparisons, several hypotheses to explain decoding have been proposed (303, 305–307). Unfortunately, experiments have not yet provided support for them or revealed any patterns of discriminatory residues in eRF1 as obvious as those apparently utilized by RF1 and RF2. A general finding of many experiments has been that stop codon recognition cannot be attributed to a single linear sequence of residues in eRF1, but likely involves more complicated structural motifs (308–311).

The Mechanism of Peptidyl-tRNA Hydrolysis

It was proposed based on the crystal structure of eRF1 that the terminal glutamine of the universally conserved GGQ motif at the tip of domain 2 might coordinate a water molecule that serves as the nucleophile for the hydrolysis of the peptidyl-tRNA ester bond (294). However, mutation of this glutamine to a glycine does not affect the ability of the mutant eRF1 to stimulate peptidyl tRNA hydrolysis (272, 294). It has also been suggested that, because they lack side chains, the glycine residues allow the entry of a water molecule into the peptidyl transferase center (289). The termination reaction is quite distinct from polypeptide elongation in that it relies on the inclusion of water in the peptidyl transferase center, whereas an elongating ribosome must employ mechanisms to keep water out during amide bond formation, lest premature peptidyl-tRNA hydrolysis occurs. On the other hand, the function of eRF1 may not simply be to provide a channel for water but to facilitate some conformational change in the ribosome that permits the entry of water into its active site as well as the activation of this water and of the ester bond, resulting in hydrolysis of the peptidyl-tRNA.

That the peptidyl transferase center catalyzes the hydrolysis reaction was supported by experiments conducted decades ago, which revealed that in the presence of 20% ethanol a deacylated tRNA or a CCA trinucleotide in the A site can stimulate peptidyl tRNA hydrolysis nearly as efficiently as *E. coli* class 1 RFs (273). The peptidyl transferase center thus appears to be activated for peptidyl tRNA hydrolysis by the GGQ motif in a manner similar to its activation for peptide elongation by tRNA, although the molecular details of this activation and the subsequent hydrolysis reaction remain unclear.

It's Not All About Factors: The Role of the Ribosome

Experiments involving eRF1s of variant genetic code organisms have suggested that the structure of eRF1 is the sole determinant of its decoding function (308, 312). Nevertheless, the evidence that the peptidyl transferase center, rather than the release factor, catalyzes peptidyl-tRNA hydrolysis implies that there must be

some communication between eRF1 and the ribosome. Work in both bacteria and eukaryotes indicates that the ribosome is not simply a bystander in the process of stop codon recognition either. Several groups have reported the isolation of mutations in both the 16S and 23S rRNAs from *E. coli* and in yeast 18S rRNA that influence the fidelity of translation termination (269, 270, 313–316; reviewed in 271, 317). For example, some mutations of base 1054 in the decoding center of yeast 18S rRNA facilitate stop codon read-through, whereas different changes to the same base compensate for the defects associated with mutant eRF1 and eRF3 proteins that themselves display omnipotent suppressor phenotypes (314). Such rRNA mutations were termed antisuppressors, because they restored fidelity to the termination reaction in the presence of the mutant RFs, suggesting that rRNA plays an important role in maintaining the efficiency of translation termination, just as it plays a central role in the decoding process during polypeptide elongation (318, 319).

Characterization of eRF3

eRF1 and eRF3 form a complex mediated by their C termini (282, 296, 297). Addition of eRF3 reduces the concentration of stop codon tetraplets required for eRF1-mediated translation termination *in vitro*. eRF3 binds GTP independently of eRF1, and stimulation of its GTPase activity requires eRF1 and the ribosome, but not the presence of a stop codon (282, 283).

The mechanism of eRF3-mediated stimulation of eRF1 activity is not known, nor is the function of GTP hydrolysis understood. The precise signal that results in the activation of GTP hydrolysis also has yet to be identified, in contrast to what has been learned about its bacterial counterpart. RF3 binds to the ribosome in its GDP-bound form and undergoes nucleotide exchange. The posttermination ribosome, still bound by RF1 or RF2 in the presence of a stop codon, was shown to be the GEF. Nucleotide exchange is activated following release of the peptide from the peptidyl-tRNA by a class 1 release factor. RF3•GTP destabilizes the binding of RFs 1 and 2, causing them to dissociate from the ribosome. GTP hydrolysis by RF3 appears to be required for its release at the conclusion of termination (278, 320). It will be interesting to determine how similar the mechanism of eRF3 is to that of RF3.

RECYCLING

The fourth stage of translation is the recycling of the ribosomal subunits so that they can be used in another round of initiation. Significant information regarding the steps in this process is available only for bacteria. At the end of the termination stage the ribosome is left on the mRNA with a deacylated tRNA, presumably in a P/E hybrid state in which the acceptor end has moved into the E site of the 50S subunit while the anticodon end remains in the P site of the 30S

subunit (321). In bacteria, this complex is recognized by ribosome release factor (RRF). RRF was initially assumed to bind in the vacant A site because of its striking structural similarity to a tRNA (322), but recent footprinting experiments have indicated that it binds across the A site, almost orthogonally to the orientation of an A-site tRNA (323). EF-G-GTP and IF3 then assist RRF in disassembling the posttermination complex (15). The exact roles of these factors are still not entirely clear, although one model (15, 323) posits that RRF in conjunction with EF-G alters the ribosome's structure to destabilize the binding of tRNA and mRNA as well as the subunit interface. IF3 then binds and facilitates complete subunit dissociation and release of the tRNA and mRNA. GTP hydrolysis by EF-G could be used actively to facilitate these disruptions or could be used to trigger release of the factors at the appropriate time.

The events and players involved in ribosome recycling in eukaryotes and archaea are largely mysterious. There does not appear to be an RRF ortholog in either kingdom. eIF1A (134), eIF3 (23, 25, 325), and eIF6 (326, 327) all have ribosome antiassociation activity *in vitro*, and eIF1 might possibly have a similar activity, given that it may be the ortholog of bacterial IF3, which is involved in recycling. However, it is not clear whether these activities are relevant for ribosome recycling or if they are instead used to prevent premature association of the subunits during initiation. Antiassociation activity is defined as shifting the equilibrium from associated to dissociated subunits, which could be important for recycling, but does not *a priori* have to be so. For ribosome recycling, a factor or factors that increase the rate of dissociation of the subunits, mRNA and deacylated tRNA would more likely be required, as is the case in the bacterial system (15). Furthermore, one might expect that factors that bind to the interface side of one of the ribosomal subunits would prevent subunit association because they sterically block joining. Whether this "activity" is relevant to the mechanism of translation, however, is another matter. For example, eIF6 has antiassociation activity and was believed to be involved in ribosome recycling (326, 327). It was later shown, however, that eIF6 is required for ribosome biogenesis, and cell extracts depleted in eIF6 did not show a defect in *in vitro* translation assays (74, 75), suggesting its antiassociation activity may not be relevant for ribosome recycling but is instead a consequence of the fact that it interacts tightly with the 60S subunit as part of its role in other pathways. [Note: Recent work has indicated that phosphorylation of eIF6 regulates the level of free 60S subunits *in vivo* (327a).]

Of the factors proposed to be involved in ribosome recycling in eukaryotes, eIF3 is perhaps the most intriguing. A variety of experiments including electron microscopic studies have suggested that eIF3 binds to the side of the 40S subunit opposite the interface (99, 139, 328). If this is so, it indicates that the antiassociation activity of the factor is due to its induction of a conformational change in the 40S subunit rather than sterically preventing binding of the 60S subunit. Inducing such a conformational change could potentially increase the rate of subunit dissociation as well as lowering the rate of association.

Finally, the closed-loop model of eukaryotic mRNAs has suggested the possibility that termination and recycling may not release the 40S subunit back into the cytoplasm. Instead, the 40S subunit may be shuttled across or over the poly(A) tail back to the 5'-end of the mRNA via the 5'- and 3'-end-associated factors. In this model, the closed loop serves to facilitate reinitiation of translation rather than (or in addition to) the first initiation event. This proposal was recently bolstered by the finding that eRF3 and PAB interact with each other (329), connecting the termination apparatus to the poly(A) tail. Overexpression of PAB was found to suppress effects associated with mutant and aberrantly folded (the prion-like PSI⁺ form) eRF3 in vivo (330), suggesting that this interaction is functionally important for termination. In addition, it was recently shown that disruption of the PAB-eRF3 interaction inhibits translation and that addition of eRF3 to in vitro translation assays stimulates the initiation process (331). Recent modeling studies have also suggested that closed-loop reinitiation could substantially increase the rate of initiation (332). At this stage, however, this model remains unproven but intriguing.

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LITERATURE CITED

1. Hershey JWB, Merrick WC. 2000. See Ref. 333, pp. 33–88
2. Kapp LD, Lorsch JR. 2004. *J. Mol. Biol.* 335:923–36
3. Kimball SR, Everson WV, Myers LM, Jefferson LS. 1987. *J. Biol. Chem.* 262: 2220–27
4. Panniers R, Rowlands AG, Henshaw EC. 1988. *J. Biol. Chem.* 263:5519–25
5. Walton GM, Gill GN. 1975. *Biochim. Biophys. Acta* 390:231–45
6. Erickson FL, Hannig EM. 1996. *EMBO J.* 15:6311–20
7. Nika J, Yang W, Pavitt GD, Hinnebusch AG, Hannig EM. 2000. *J. Biol. Chem.* 275:26011–17
8. Schmitt E, Blanquet S, Mechulam Y. 2002. *EMBO J.* 21:1821–32
9. Lorsch JR, Herschlag D. 1999. *EMBO J.* 18:6705–17
10. Moazed D, Samaha RR, Gualerzi C, Noller HF. 1995. *J. Mol. Biol.* 248: 207–10
11. Carter AP, Clemons WM Jr, Brodersen DE, Morgan-Warren RJ, Hartsch T, et al. 2001. *Science* 291:498–501
12. Gualerzi CO, Pon CL. 1990. *Biochemistry* 29:5881–89

13. Luchin S, Putzer H, Hershey JW, Ceniempo Y, Grunberg-Manago M, Laalami S. 1999. *J. Biol. Chem.* 274: 6074–79
14. Grunberg-Manago M, Dessen P, Pantalononi D, Godefroy-Colburn T, Wolfe AD, Dondon J. 1975. *J. Mol. Biol.* 94: 461–78
15. Karimi R, Pavlov MY, Buckingham RH, Ehrenberg M. 1999. *Mol. Cell* 3: 601–9
16. Kyrpides NC, Woese CR. 1998. *Proc. Natl. Acad. Sci. USA* 95:224–28
17. Choi SK, Lee JH, Zoll WL, Merrick WC, Dever TE. 1998. *Science* 280: 1757–60
18. Lee JH, Choi SK, Roll-Mecak A, Burley SK, Dever TE. 1999. *Proc. Natl. Acad. Sci. USA* 96:4342–47
19. Sorensen HP, Hedegaard J, Sperling-Petersen HU, Mortensen KK. 2001. *IUBMB Life* 51:321–27
20. Dever TE. 2002. *Cell* 108:545–56
21. Pestova TV, Kolupaeva VG. 2002. *Genes Dev.* 16:2906–22
22. Goumans H, Thomas A, Verhoeven A, Voorma HO, Benne R. 1980. *Biochim. Biophys. Acta* 608:39–46
23. Goss DJ, Rounds DJ. 1988. *Biochemistry* 27:3610–13
24. Nakaya K, Ranu RS, Wool IG. 1973. *Biochem. Biophys. Res. Commun.* 54: 246–55
25. Goss DJ, Rounds D, Harrigan T, Woodley CL, Wahba AJ. 1988. *Biochemistry* 27:1489–94
26. Gualerzi C, Wintermeyer W. 1986. *FEBS Lett.* 202:1–6
27. Szkaradkiewicz K, Zuleeg T, Limmer S, Sprinzl M. 2000. *Eur. J. Biochem.* 267:4290–99
28. Roll-Mecak A, Cao C, Dever TE, Burley SK. 2000. *Cell* 103:781–92
29. Shin B-S, Maag D, Roll-Mecak A, Arefin MS, Burley SK, et al. 2002. *Cell* 111: 1015–25
30. Pestova TV, Lomakin IB, Lee JH, Choi SK, Dever TE, Hellen CU. 2000. *Nature* 403:332–35
31. Cui Y, Dinman JD, Kinzy TG, Peltz SW. 1998. *Mol. Cell Biol.* 18:1506–16
32. Yoon HJ, Donahue TF. 1992. *Mol. Cell Biol.* 12:248–60
33. Keeling PJ, Doolittle WF. 1995. *Proc. Natl. Acad. Sci. USA* 92:5761–64
34. Huang H, Yoon H, Hannig EM, Donahue TF. 1997. *Genes Dev.* 11: 2396–413
35. Phan L, Zhang XL, Asano K, Anderson J, Vornlocher HP, et al. 1998. *Mol. Cell Biol.* 18:4935–46
36. Merrick WC. 1979. *Methods Enzymol.* 60:108–23
37. Merrick WC. 1979. *Methods Enzymol.* 60:101–6
38. Trachsel H, Ermi B, Schreier MH, Staehelin T. 1977. *J. Mol. Biol.* 116:755–67
39. Benne R, Hershey JW. 1976. *Proc. Natl. Acad. Sci. USA* 73:3005–9
40. Burks EA, Bezerra PP, Le H, Gallie DR, Browning KS. 2001. *J. Biol. Chem.* 276:2122–31
41. Benne R, Hershey JWB. 1978. *J. Biol. Chem.* 253:3078–87
42. Valasek L, Nielsen KH, Hinnebusch AG. 2002. *EMBO J.* 21:5886–98
43. Chaudhuri J, Chowdhury D, Maitra U. 1999. *J. Biol. Chem.* 273:17975–80
44. Phan L, Schoenfeld LW, Valasek L, Nielsen KH, Hinnebusch AG. 2001. *EMBO J.* 20:2954–65
45. Jackson RJ. 1996. See Ref. 334, pp. 71–112
46. Kozak M. 2002. *Gene* 299:1–34
47. Ishigaki Y, Li XJ, Serin G, Maquat LE. 2001. *Cell* 106:607–17
48. Linder P. 1992. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 62:47–62
49. Grifo JA, Tahara SM, Leis JP, Morgan MA, Shatkin AJ, Merrick WC. 1982. *J. Biol. Chem.* 257:5246–52
50. Rozen F, Edery I, Meerovitch K, Dever TE, Merrick WC, Sonenberg N. 1990. *Mol. Cell Biol.* 10:1134–44
51. Lawson TG, Lee KA, Maimone MM,

- Abramson RD, Dever TE, et al. 1989. *Biochemistry* 28:4729–34
52. Ray BK, Lawson TG, Kramer JC, Cladaras MH, Grifo JA, et al. 1985. *J. Biol. Chem.* 260:7651–58
53. Coppolecchia R, Buser P, Stotz A, Linder P. 1993. *EMBO J.* 12:4005–11
54. Altmann M, Muller PP, Wittmer B, Ruchti F, Lanker S, Trachsel H. 1993. *EMBO J.* 12:3997–4003
55. Richter-Cook NJ, Dever TE, Hensold JO, Merrick WC. 1998. *J. Biol. Chem.* 273:7579–87
56. Richter NJ, Rogers GW Jr, Hensold JO, Merrick WC. 1999. *J. Biol. Chem.* 274:35415–24
57. Rogers GW Jr, Richter NJ, Lima WF, Merrick WC. 2001. *J. Biol. Chem.* 276:30914–22
58. Hentze MW. 1997. *Science* 275:500–1
59. Grifo JA, Tahara SM, Morgan MA, Shatkin AJ, Merrick WC. 1983. *J. Biol. Chem.* 258:5804–10
60. Tarun SZ Jr, Sachs AB. 1996. *EMBO J.* 15:7168–77
61. Tarun SZ Jr, Sachs AB. 1995. *Genes Dev.* 9:2997–3007
62. Gallie DR. 1991. *Genes Dev.* 5:2108–16
63. Preiss T, Hentze MW. 1998. *Nature* 392:516–20
64. Munroe D, Jacobson A. 1990. *Mol. Cell Biol.* 10:3441–55
65. Withey JH, Friedman DI. 2003. *Annu. Rev. Microbiol.* 57:101–23
66. Benne R, Brown-Luedi ML, Hershey JW. 1978. *J. Biol. Chem.* 253:3070–77
67. Kemper WM, Berry KW, Merrick WC. 1976. *J. Biol. Chem.* 251:5551–57
68. Kang HA, Hershey JW. 1994. *J. Biol. Chem.* 269:3934–40
69. Zuk D, Jacobson A. 1998. *EMBO J.* 17:2914–25
70. Valentini SR, Casolari JM, Oliveira CC, Silver PA, McBride AE. 2002. *Genetics* 160:393–405
71. Zoll WL, Horton LE, Komar AA, Hensold JO, Merrick WC. 2002. *J. Biol. Chem.* 277:37079–87
72. Chakravarty I, Bagchi MK, Roy R, Banerjee AC, Gupta NK. 1985. *J. Biol. Chem.* 260:6945–49
73. Tyzack JK, Wang X, Belsham GJ, Proud CG. 2000. *J. Biol. Chem.* 275:34131–39
74. Si K, Maitra U. 1999. *Mol. Cell Biol.* 19:1416–26
75. Basu U, Si K, Warner JR, Maitra U. 2001. *Mol. Cell Biol.* 21:1453–62
76. Hutvagner G, Zamore PD. 2002. *Science* 297:2056–60
77. Dennis PP. 1997. *Cell* 89:1007–10
78. Ramesh V, RajBhandary UL. 2001. *J. Biol. Chem.* 276:3660–65
79. Keeling PJ, Doolittle WF. 1995. *Mol. Microbiol.* 17:399–400
80. Kyrpides NC, Woese CR. 1998. *Proc. Natl. Acad. Sci. USA* 95:3726–30
81. Kurzchalia TV, Bommer UA, Babkina GT, Karpova GG. 1984. *FEBS Lett.* 175:313–16
82. Pavitt GD, Ramaiah KV, Kimball SR, Hinnebusch AG. 1998. *Genes Dev.* 12:514–26
83. Lecompte O, Ripp R, Thierry JC, Moras D, Poch O. 2002. *Nucleic Acids Res.* 30:5382–90
84. Ganoza MC, Kiel MC, Aoki H. 2002. *Microbiol. Mol. Biol. Rev.* 66:460–85
85. Tanner NK, Linder P. 2001. *Mol. Cell* 8:251–62
86. Kressler D, de la Cruz J, Rojo M, Linder P. 1997. *Mol. Cell Biol.* 17:7283–94
87. Lu J, Aoki H, Ganoza MC. 1999. *Int. J. Biochem. Cell Biol.* 31:215–29
88. Moll I, Grill S, Grundling A, Blasi U. 2002. *Mol. Microbiol.* 44:1387–96
89. Parkhurst KM, Hileman RE, Saha D, Gupta NK, Parkhurst LJ. 1994. *Biochemistry* 33:15168–77
90. Hinnebusch AG. 2000. See Ref. 333, pp. 185–244
91. Dasso MC, Milburn SC, Hershey JW,

- Jackson RJ. 1990. *Eur. J. Biochem.* 187: 361–71
92. Naranda T, MacMillan SE, Donahue TF, Hershey JW. 1996. *Mol. Cell Biol.* 16:2307–13
93. Olsen DS, Savner EM, Mathew A, Zhang F, Krishnamoorthy T, et al. 2003. *EMBO J.* 22:193–204
94. Methot N, Song MS, Sonenberg N. 1996. *Mol. Cell Biol.* 16:5328–34
95. Vornlocher HP, Hanachi P, Ribeiro S, Hershey JW. 1999. *J. Biol. Chem.* 274: 16802–12
96. Imataka H, Sonenberg N. 1997. *Mol. Cell Biol.* 17:6940–47
97. Asano K, Shalev A, Phan L, Nielsen K, Clayton J, et al. 2001. *EMBO J.* 20: 2326–37
98. Peterson DT, Merrick WC, Safer B. 1979. *J. Biol. Chem.* 254:2509–16
99. Valasek L, Mathew AA, Shin BS, Nielsen KH, Szamecz B, Hinnebusch AG. 2003. *Genes Dev.* 17:786–99
100. Pincheira R, Chen Q, Huang Z, Zhang JT. 2001. *Eur. J. Cell Biol.* 80:410–18
101. Hasek J, Kovarik P, Valasek L, Malinska K, Schneider J, et al. 2000. *Cell Motil. Cytoskelet.* 45:235–46
102. Hou CL, Tang C, Roffler SR, Tang TK. 2000. *Blood* 96:747–53
103. Palecek J, Hasek J, Ruis H. 2001. *Biochem. Biophys. Res. Commun.* 282: 1244–50
104. Lin L, Holbro T, Alonso G, Gerosa D, Burger MM. 2001. *J. Cell Biochem.* 80: 483–90
105. von Arnim AG, Chamovitz DA. 2003. *Curr. Biol.* 13:R323–25
106. Asano K, Clayton J, Shalev A, Hinnebusch AG. 2000. *Genes Dev.* 14: 2534–46
107. Valasek L, Phan L, Schoenfeld LW, Valaskova V, Hinnebusch AG. 2001. *EMBO J.* 20:891–904
108. Stevens SW, Ryan DE, Ge HY, Moore RE, Young MK, et al. 2002. *Mol. Cell* 9:31–44
109. Ellis RJ. 2001. *Curr. Opin. Struct. Biol.* 11:114–19
110. von der Haar T, McCarthy JEG. 2002. *Mol. Microbiol.* 46:531–44
111. He H, von der Haar T, Singh CR, Li M, Li B, et al. 2003. *Mol. Cell Biol.* 23: 5431–45
112. Asano K, Krishnamoorthy T, Phan L, Pavitt GD, Hinnebusch AG. 1999. *EMBO J.* 18:1673–88
113. von der Haar T, Ball PD, McCarthy JEG. 2000. *J. Biol. Chem.* 275: 30551–55
114. Wei C, Balasta ML, Ren J, Goss DJ. 1998. *Biochemistry* 37:1910–16
115. Luo Y, Goss DJ. 2001. *J. Biol. Chem.* 276:43083–86
116. Korneeva NL, Lamphear BJ, Hennigan FL, Rhoads RE. 2000. *J. Biol. Chem.* 275:41369–76
117. Maag D, Lorsch JR. 2003. *J. Mol. Biol.* 330:917–24
118. Marcotrigiano J, Lomakin IB, Sonenberg N, Pestova TV, Hellen CU, Burley SK. 2001. *Mol. Cell* 7:193–203
119. Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK. 1999. *Mol. Cell* 3: 707–16
120. Hershey PEC, McWhirter SM, Gross JD, Wagner G, Alber T, Sachs AB. 1999. *J. Biol. Chem.* 274:21297–304
121. Fletcher CM, Wagner G. 1998. *Protein Sci.* 7:1639–42
122. Fletcher CM, McGuire AM, Gingras AC, Li H, Matsuo H, et al. 1998. *Biochemistry* 37:9–15
123. Choi SK, Olsen DS, Roll-Mecak A, Martung A, Remo KL, et al. 2000. *Mol. Cell Biol.* 20:7183–91
124. Marintchev A, Kolupaeva VG, Pestova TV, Wagner G. 2003. *Proc. Natl. Acad. Sci. USA* 100:1535–40
125. Battiste JL, Pestova TV, Hellen CU, Wagner G. 2000. *Mol. Cell* 5:109–19
126. Fletcher CM, Pestova TV, Hellen CU, Wagner G. 1999. *EMBO J.* 18:2631–37
127. Naranda T, Sirangelo I, Fabbri BJ, Hershey JW. 1995. *FEBS Lett.* 372:249–52

128. Astrom SU, von Pawel-Rammingen U, Bystrom AS. 1993. *J. Mol. Biol.* 233: 43–58
129. Farruggio D, Chaudhuri J, Maitra U, Rajbhandary UL. 1996. *Mol. Cell Biol.* 16:4248–56
130. von Pawel-Rammingen U, Astrom S, Bystrom AS. 1992. *Mol. Cell Biol.* 12: 1432–42
131. Forster C, Chakraborty K, Sprinzl M. 1993. *Nucleic Acids Res.* 21:5679–83
132. Astrom SU, Bystrom AS. 1994. *Cell* 79: 535–46
133. Drabkin HJ, Estrella M, Rajbhandary UL. 1998. *Mol. Cell Biol.* 18:1459–66
134. Thomas A, Goumans H, Voorma HO, Benne R. 1980. *Eur. J. Biochem.* 107: 39–45
135. Chaudhuri J, Si K, Maitra U. 1997. *J. Biol. Chem.* 272:7883–91
136. Majumdar R, Bandyopadhyay A, Maitra U. 2003. *J. Biol. Chem.* 278: 6580–87
137. Algire MA, Maag D, Savio P, Acker MG, Tarun SZ Jr, et al. 2002. *RNA* 8: 382–97
138. Hinnebusch AG. 1996. See Ref. 334, pp. 199–244
139. Bommer UA, Lutsch G, Stahl J, Bielka H. 1991. *Biochimie* 73:1007–19
140. Bommer UA, Lutsch G, Behlke J, Stahl J, Nesytova N, et al. 1988. *Eur. J. Biochem.* 172:653–62
141. Bommer UA, Stahl J, Henske A, Lutsch G, Bielka H. 1988. *FEBS Lett.* 233: 114–18
142. Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK. 1997. *Cell* 89: 951–61
143. Tomoo K, Shen X, Okabe K, Nozoe Y, Fukuhara S, et al. 2002. *Biochem. J.* 362: 539–44
144. Matsuo H, McGuire AM, Fletcher CM, Gingras AC, Sonenberg N, Wagner G. 1997. *Nat. Struct. Biol.* 4:717–24
145. Iizuka N, Najita L, Franzusoff A, Sarnow P. 1994. *Mol. Cell Biol.* 14:7322–30
146. Michel YM, Poncet D, Piron M, Kean KM, Borman AM. 2000. *J. Biol. Chem.* 275:32268–76
147. Svitkin YV, Ovchinnikov LP, Dreyfuss G, Sonenberg N. 1996. *EMBO J.* 15: 7147–55
148. Lang V, Zanchin NI, Lunsdorf H, Tuite M, McCarthy JE. 1994. *J. Biol. Chem.* 269:6117–23
149. Vasilescu S, Ptushkina M, Linz B, Muller PP, McCarthy JE. 1996. *J. Biol. Chem.* 271:7030–37
150. Niedzwiecka A, Marcotrigiano J, Stepinski J, Jankowska-Anyszka M, Wyslouch-Cieszynska A, et al. 2002. *J. Mol. Biol.* 319:615–35
151. Vega Laso MR, Zhu D, Sagliocco F, Brown AJ, Tuite MF, McCarthy JE. 1993. *J. Biol. Chem.* 268:6453–62
152. Pelletier J, Sonenberg N. 1985. *Cell* 40: 515–26
153. Kozak M. 1986. *Proc. Natl. Acad. Sci. USA* 83:2850–54
154. Baim SB, Sherman F. 1988. *Mol. Cell Biol.* 8:1591–601
155. Kozak M. 1989. *Mol. Cell Biol.* 9: 5134–42
156. Lee KA, Guertin D, Sonenberg N. 1983. *J. Biol. Chem.* 258:707–10
157. Lawson TG, Ray BK, Dodds JT, Grifo JA, Abramson RD, et al. 1986. *J. Biol. Chem.* 261:13979–89
158. Muckenthaler M, Gray NK, Hentze MW. 1998. *Mol. Cell* 2:383–88
159. Lamphear BJ, Kirchweger R, Skern T, Rhoads RE. 1995. *J. Biol. Chem.* 270: 21975–83
160. Svitkin YV, Pause A, Haghghat A, Pyronnet S, Witherell G, et al. 2001. *RNA* 7:382–94
161. Grifo JA, Abramson RD, Satler CA, Merrick WC. 1984. *J. Biol. Chem.* 259: 8648–54
162. Pause A, Sonenberg N. 1992. *EMBO J.* 11:2643–54
163. Blum S, Schmid SR, Pause A, Buser P, Linder P, et al. 1992. *Proc. Natl. Acad. Sci. USA* 89:7664–68

164. Lorsch JR, Herschlag D. 1998. *Biochemistry* 37:2180–93
165. Lorsch JR, Herschlag D. 1998. *Biochemistry* 37:2194–206
166. Johnson ER, McKay DB. 1999. *RNA* 5: 1526–34
167. Caruthers JM, Johnson ER, McKay DB. 2000. *Proc. Natl. Acad. Sci. USA* 97: 13080–85
168. Benz J, Trachsel H, Baumann U. 1999. *Struct. Fold. Des.* 7:671–79
169. Pause A, Methot N, Sonenberg N. 1993. *Mol. Cell Biol.* 13:6789–98
170. Rogers GW Jr, Lima WF, Merrick WC. 2001. *J. Biol. Chem.* 276:12598–608
171. Rogers GW Jr, Richter NJ, Merrick WC. 1999. *J. Biol. Chem.* 274: 12236–44
172. Lohman TM, Bjornson KP. 1996. *Annu. Rev. Biochem.* 65:169–214
173. Abramson RD, Dever TE, Merrick WC. 1988. *J. Biol. Chem.* 263:6016–19
174. Bi X, Ren J, Goss DJ. 2000. *Biochemistry* 39:5758–65
175. Berset C, Zurbriggen A, Djafarzadeh S, Altmann M, Trachsel H. 2003. *RNA* 9: 871–80
176. Sonenberg N. 1988. *Prog. Nucleic Acid Res. Mol. Biol.* 35:173–207
177. Duncan R, Milburn SC, Hershey JW. 1987. *J. Biol. Chem.* 262:380–88
178. Pollard TD, Cooper JA. 1986. *Annu. Rev. Biochem.* 55:987–1035
179. Pause A, Methot N, Svitkin Y, Merrick WC, Sonenberg N. 1994. *EMBO J.* 13: 1205–15
180. Altmann M, Wittmer B, Methot N, Sonenberg N, Trachsel H. 1995. *EMBO J.* 14:3820–27
181. Pisarev AV, Skabkin MA, Thomas AA, Merrick WC, Ovchinnikov LP, Shatsky IN. 2002. *J. Biol. Chem.* 277:15445–51
182. Iost I, Dreyfus M, Linder P. 1999. *J. Biol. Chem.* 274:17677–83
183. Chuang RY, Weaver PL, Liu Z, Chang TH. 1997. *Science* 275:1468–71
184. de la Cruz J, Iost I, Kressler D, Linder P. 1997. *Proc. Natl. Acad. Sci. USA* 94: 5201–6
185. Verlhac MH, Chen RH, Hanachi P, Hershey JW, Derynck R. 1997. *EMBO J.* 16:6812–22
186. Naranda T, MacMillan SE, Hershey JW. 1994. *J. Biol. Chem.* 269: 32286–92
187. Deo RC, Bonanno JB, Sonenberg N, Burley SK. 1999. *Cell* 98:835–45
188. Baer BW, Kornberg RD. 1980. *Proc. Natl. Acad. Sci. USA* 77:1890–92
189. Baer BW, Kornberg RD. 1983. *J. Cell Biol.* 96:717–21
190. Kessler SH, Sachs AB. 1998. *Mol. Cell Biol.* 18:51–57
191. Gray NK, Collier JM, Dickson KS, Wickens M. 2000. *EMBO J.* 19: 4723–33
192. Wells SE, Hillner PE, Vale RD, Sachs AB. 1998. *Mol. Cell* 2:135–40
193. Borman AM, Michel YM, Kean KM. 2000. *Nucleic Acids Res.* 28:4068–75
194. Borman AM, Michel YM, Malnou CE, Kean KM. 2002. *J. Biol. Chem.* 277: 36818–24
195. Bi XP, Goss DJ. 2000. *J. Biol. Chem.* 275:17740–46
196. Herschlag D, Johnson FB. 1993. *Genes Dev.* 7:173–79
197. Proweller A, Butler JS. 1997. *J. Biol. Chem.* 272:6004–10
198. Munroe D, Jacobson A. 1990. In *The Ribosome*, ed. WE Hill, A Dahlberg, RA Garrett, PB Moore, D Schlessinger, JR Warner, pp. 299–305. Washington, DC: ASM Press
199. Jacobson A. 1996. See Ref. 334, pp. 459–80
200. Sachs AB, Davis RW. 1989. *Cell* 58: 857–67
201. Proweller A, Butler JS. 1996. *J. Biol. Chem.* 271:10859–65
202. Searfoss A, Dever TE, Wickner R. 2001. *Mol. Cell Biol.* 21:4900–8
203. Searfoss AW, Wickner RB. 2000. *Proc. Natl. Acad. Sci. USA* 97:9133–37

204. Otero LJ, Ashe MP, Sachs AB. 1999. *EMBO J.* 18:3153–63
205. Craig AW, Haghighat A, Yu AT, Sonenberg N. 1998. *Nature* 392: 520–23
206. Paraskeva E, Gray NK, Schlager B, Wehr K, Hentze MW. 1999. *Mol. Cell Biol.* 19:807–16
207. Hanson S, Berthelot K, Fink B, McCarthy JEG, Suess B. 2003. *Mol. Microbiol.* 49:1627–37
208. Pestova TV, Borukhov SI, Hellen CUT. 1998. *Nature* 394:854–59
209. Kozak M. 1980. *Cell* 22:459–67
210. Cigan AM, Feng L, Donahue TF. 1988. *Science* 242:93–97
211. Donahue TF, Cigan AM, Pabich EK, Valavicius BC. 1988. *Cell* 54:621–32
212. Cigan AM, Pabich EK, Feng L, Donahue TF. 1989. *Proc. Natl. Acad. Sci. USA* 86:2784–88
213. Castilho-Valavicius B, Yoon H, Donahue TF. 1990. *Genetics* 124: 483–95
214. Hashimoto NN, Carnevalli LS, Castilho BA. 2002. *Biochem. J.* 367:359–68
215. Ogle JM, Brodersen DE, Clemons WM Jr, Tarry MJ, Carter AP, Ramakrishnan V. 2001. *Science* 292:902
216. Dallas A, Noller HF. 2001. *Mol. Cell* 8:855–64
- 216a. Lomakin IB, Kolupaeva VG, Marintchev A, Wagner G, Pestova TV. 2003. *Genes Dev.* 17:2786–97
217. Piepenburg O, Pape T, Pleiss JA, Wintermeyer W, Uhlenbeck OC, Rodnina MV. 2000. *Biochemistry* 39:1734–38
218. Yarus M, Smith D. 1995. In *tRNA: Structure, Biosynthesis and Function*, ed. D Soll, UC Rajbhandary, pp. 443–70. Washington, DC: ASM Press
219. Das S, Maitra U. 2001. *Prog. Nucleic Acid Res. Mol. Biol.* 70:207–31
220. Das S, Maitra U. 2000. *Mol. Cell Biol.* 20:3942–50
221. Paulin FE, Campbell LE, O'Brien K, Loughlin J, Proud CG. 2001. *Curr. Biol.* 11:55–59
222. Kozak M. 1994. *Biochimie* 76:815–21
223. Cigan AM, Donahue TF. 1987. *Gene* 59: 1–18
224. Hamilton R, Watanabe CK, de Boer HA. 1987. *Nucleic Acids Res.* 15: 3581–93
225. Joshi CP, Zhou H, Huang X, Chiang VL. 1997. *Plant Mol. Biol.* 35: 993–1001
226. Yun DF, Laz TM, Clements JM, Sherman F. 1996. *Mol. Microbiol.* 19: 1225–39
227. Donahue TF, Cigan AM. 1988. *Mol. Cell Biol.* 8:2955–63
228. Looman AC, Kuivenhoven JA. 1993. *Nucleic Acids Res.* 21:4268–71
229. Cigan AM, Pabich EK, Donahue TF. 1988. *Mol. Cell Biol.* 8:2964–75
230. Slusher LB, Gillman EC, Martin NC, Hopper AK. 1991. *Proc. Natl. Acad. Sci. USA* 88:9789–93
231. Peri S, Pandey A. 2001. *Trends Genet.* 17:685–87
232. Kozak M. 2000. *Genomics* 70:396–406
233. Merrick WC. 1979. *J. Biol. Chem.* 254: 3708–11
234. Lee JH, Pestova TV, Shin BS, Cao C, Choi SK, Dever TE. 2002. *Proc. Natl. Acad. Sci. USA* 99:16689–94
235. Merrick WC, Kemper WM, Anderson WF. 1975. *J. Biol. Chem.* 250:5556–62
236. Rodnina MV, Savelsbergh A, Katunin VI, Wintermeyer W. 1997. *Nature* 385: 37–41
237. Ramakrishnan V. 2002. *Cell* 108: 557–72
238. Spahn CM, Beckmann R, Eswar N, Penczek PA, Sali A, et al. 2001. *Cell* 107:373–86
239. Rodnina MV, Wintermeyer W. 2001. *Annu. Rev. Biochem.* 70:415–35
240. Moore PB, Steitz TA. 2003. *RNA* 9: 155–59
241. Green R, Noller HF. 1997. *Annu. Rev. Biochem.* 66:679–716
242. Wintermeyer W, Savelsbergh A, Semenov YP, Katunin VI, Rodnina

- MV. 2001. *Cold Spring Harbor Symp. Quant. Biol.* 66:449–58
243. Cavallius J, Zoll W, Chakraborty K, Merrick WC. 1993. *Biochim. Biophys. Acta* 1163:75–80
244. Fujita T, Itoh T. 1995. *Biochem. Mol. Biol. Int.* 37:107–15
245. De Vendittis E, Amatruda MR, Masullo M, Bocchini V. 1993. *Gene* 136:41–48
246. Andersen GR, Valente L, Pedersen L, Kinzy TG, Nyborg J. 2001. *Nat. Struct. Biol.* 8:531–34
247. Valle M, Sengupta J, Swami NK, Grassucci RA, Burkhardt N, et al. 2002. *EMBO J.* 21:3557–67
248. Stark H, Rodnina MV, Wieden HJ, Zemlin F, Wintermeyer W, van Heel M. 2002. *Nat. Struct. Biol.* 9:849–54
249. Jorgensen R, Ortiz PA, Carr-Schmid A, Nissen P, Kinzy TG, Andersen GR. 2003. *Nat. Struct. Biol.* 10:379–85
250. Dever TE, Costello CE, Owens CL, Rosenberry TL, Merrick WC. 1989. *J. Biol. Chem.* 264:20518–25
251. Van Ness BG, Howard JB, Bodley JW. 1978. *J. Biol. Chem.* 253:8687–90
252. Skogerson L, Engelhardt D. 1977. *J. Biol. Chem.* 252:1471–75
253. Qin SL, Xie AG, Bonato MC, McLaughlin CS. 1990. *J. Biol. Chem.* 265:1903–12
254. Dasmahapatra B, Chakraborty K. 1981. *J. Biol. Chem.* 256:9999–10004
255. Kovalchuk O, Kambampati R, Pladies E, Chakraborty K. 1998. *Eur. J. Biochem.* 258:986–93
256. Anand M, Chakraborty K, Marton MJ, Hinnebusch AG, Kinzy TG. 2003. *J. Biol. Chem.* 278:6985–91
257. Triana-Alonso FJ, Chakraborty K, Nierhaus KH. 1995. *J. Biol. Chem.* 270:20473–78
258. Rodnina MV, Serebryanik AI, Ovcharenko GV, El'Skaya AV. 1994. *Eur. J. Biochem.* 225:305–10
259. El'Skaya AV, Ovcharenko GV, Palchevskii SS, Petrusenko ZM, Triana-Alonso FJ, Nierhaus KH. 1997. *Biochemistry* 36:10492–97
260. Kiel MC, Ganoza MC. 2001. *Eur. J. Biochem.* 268:278–86
261. Kiel MC, Aoki H, Ganoza MC. 1999. *Biochimie* 81:1097–108
262. Inge-Vechtomov S, Zhouravleva G, Philippe M. 2003. *Biol. Cell* 95:195–209
263. Kisselev L, Ehrenberg M, Frolova L. 2003. *EMBO J.* 22:175–82
264. Nakamura Y, Ito K. 2003. *Trends Biochem. Sci.* 28:99–105
265. Bertram G, Innes S, Minella O, Richardson J, Stansfield I. 2001. *Microbiology* 147:255–69
266. Kisselev LL, Buckingham RH. 2000. *Trends Biochem. Sci.* 25:561–66
267. Poole E, Tate W. 2000. *Biochim. Biophys. Acta* 1493:1–11
268. Caskey CT, Beaudet AL, Scolnick EM, Rosman M. 1971. *Proc. Natl. Acad. Sci. USA* 68:3163–67
269. Arkov AL, Freistoffer DV, Ehrenberg M, Murgola EJ. 1998. *EMBO J.* 17:1507–14
270. Arkov AL, Hedenstierna KO, Murgola EJ. 2002. *J. Bacteriol.* 184:5052–57
271. Arkov AL, Murgola EJ. 1999. *Biochemistry* 38:1354–59
272. Seit-Nebi A, Frolova L, Justesen J, Kisselev L. 2001. *Nucleic Acids Res.* 29:3982–87
273. Zavialov AV, Mora L, Buckingham RH, Ehrenberg M. 2002. *Mol. Cell* 10:789–98
274. Scolnick E, Tompkins R, Caskey T, Nirenberg M. 1968. *Proc. Natl. Acad. Sci. USA* 61:768–74
275. Ito K, Uno M, Nakamura Y. 2000. *Nature* 403:680–84
276. Grentzmann G, Brechemier-Baey D, Heurgue V, Mora L, Buckingham RH. 1994. *Proc. Natl. Acad. Sci. USA* 91:5848–52
277. Mikuni O, Ito K, Moffat J, Matsumura K, McCaughan K, et al. 1994. *Proc. Natl. Acad. Sci. USA* 91:5798–802

278. Freistroffer DV, Pavlov MY, MacDougall J, Buckingham RH, Ehrenberg M. 1997. *EMBO J.* 16:4126–33
279. Konecki DS, Aune KC, Tate W, Caskey CT. 1977. *J. Biol. Chem.* 252:4514–20
280. Frolova L, Le Goff X, Rasmussen HH, Cheperegin S, Drugeon G, et al. 1994. *Nature* 372:701–3
281. Dontsova M, Frolova L, Vassilieva J, Piendl W, Kisselev L, Garber M. 2000. *FEBS Lett.* 472:213–16
282. Zhouravleva G, Frolova L, Le Goff X, Le Guellec R, Inge-Vechtomov S, et al. 1995. *EMBO J.* 14:4065–72
283. Frolova L, Le Goff X, Zhouravleva G, Davydova E, Philippe M, Kisselev L. 1996. *RNA* 2:334–41
284. Wilson PG, Culbertson MR. 1988. *J. Mol. Biol.* 199:559–73
285. Stansfield I, Jones KM, Kushnirov VV, Dagkesamanskaya AR, Poznyakovski AI, et al. 1995. *EMBO J.* 14:4365–73
286. Le Goff X, Philippe M, Jean-Jean O. 1997. *Mol. Cell Biol.* 17:3164–72
287. Ito K, Ebihara K, Uno M, Nakamura Y. 1996. *Proc. Natl. Acad. Sci. USA* 93:5443–48
288. Pel HJ, Moffat JG, Ito K, Nakamura Y, Tate WP. 1998. *RNA* 4:47–54
289. Frolova LY, Tsvikovskii RY, Sivolobova GF, Oparina NY, Serpinsky OI, et al. 1999. *RNA* 5:1014–20
290. Brown CM, Stockwell PA, Trotman CN, Tate WP. 1990. *Nucleic Acids Res.* 18:6339–45
291. Brown CM, Stockwell PA, Trotman CN, Tate WP. 1990. *Nucleic Acids Res.* 18:2079–86
292. Poole ES, Major LL, Mannering SA, Tate WP. 1998. *Nucleic Acids Res.* 26:954–60
293. Ozawa Y, Hanaoka S, Saito R, Washio T, Nakano S, et al. 2002. *Gene* 300:79–87
294. Song H, Mugnier P, Das AK, Webb HM, Evans DR, et al. 2000. *Cell* 100:311–21
295. Frolova LY, Merkulova TI, Kisselev LL. 2000. *RNA* 6:381–90
296. Merkulova TI, Frolova LY, Lazar M, Camonis J, Kisselev LL. 1999. *FEBS Lett.* 443:41–47
297. Eurwilaichitr L, Graves FM, Stansfield I, Tuite MF. 1999. *Mol. Microbiol.* 32:485–96
298. Vestergaard B, Van LB, Andersen GR, Nyborg J, Buckingham RH, Kjeldgaard M. 2001. *Mol. Cell* 8:1375–82
299. Klaholz BP, Pape T, Zavialov AV, Myasnikov AG, Orlova EV, et al. 2003. *Nature* 421:90–94
300. Rawat UB, Zavialov AV, Sengupta J, Valle M, Grassucci RA, et al. 2003. *Nature* 421:87–90
301. Scarlett DJ, McCaughan KK, Wilson DN, Tate WP. 2003. *J. Biol. Chem.* 278:15095–104
302. Bertram G, Bell HA, Ritchie DW, Fullerton G, Stansfield I. 2000. *RNA* 6:1236–47
303. Lozupone CA, Knight RD, Landweber LF. 2001. *Curr. Biol.* 11:65–74
304. Karamyshev AL, Ito K, Nakamura Y. 1999. *FEBS Lett.* 457:483–88
305. Inagaki Y, Doolittle WF. 2001. *Nucleic Acids Res.* 29:921–27
306. Inagaki Y, Blouin C, Doolittle WF, Roger AJ. 2002. *Nucleic Acids Res.* 30:532–44
307. Muramatsu T, Heckmann K, Kitanaka C, Kuchino Y. 2001. *FEBS Lett.* 488:105–9
308. Ito K, Frolova L, Seit-Nebi A, Karamyshev A, Kisselev L, Nakamura Y. 2002. *Proc. Natl. Acad. Sci. USA* 99:8494–99
309. Frolova L, Seit-Nebi A, Kisselev L. 2002. *RNA* 8:129–36
310. Seit-Nebi A, Frolova L, Kisselev L. 2002. *EMBO Rep.* 3:881–86
311. Chavatte L, Kervestin S, Favre A, Jean-Jean O. 2003. *EMBO J.* 22:1644–53
312. Kervestin S, Frolova L, Kisselev L, Jean-Jean O. 2001. *EMBO Rep.* 2:680–84
313. Velichutina IV, Dresios J, Hong JY, Li

- C, Mankin A, et al. 2000. *RNA* 6: 1174–84
314. Chernoff YO, Newnam GP, Liebman SW. 1996. *Proc. Natl. Acad. Sci. USA* 93:2517–22
315. Chernoff YO, Vincent A, Liebman SW. 1994. *EMBO J.* 13:906–13
316. Velichutina IV, Hong JY, Mesecar AD, Chernoff YO, Liebman SW. 2001. *J. Mol. Biol.* 305:715–27
317. Liebman SW, Chernoff YO, Liu R. 1995. *Biochem. Cell Biol.* 73:1141–49
318. Ogle JM, Brodersen DE, Clemons WM Jr, Tarry MJ, Carter AP, Ramakrishnan V. 2001. *Science* 292:897–902
319. Ogle JM, Murphy FV, Tarry MJ, Ramakrishnan V. 2002. *Cell* 111: 721–32
320. Zavialov AV, Buckingham RH, Ehrenberg M. 2001. *Cell* 107:115–24
321. Moazed D, Noller HF. 1989. *Nature* 342: 142–48
322. Selmer M, Al-Karadaghi S, Hirokawa G, Kaji A, Liljas A. 1999. *Science* 286: 2349–52
323. Lancaster L, Kiel MC, Kaji A, Noller HF. 2002. *Cell* 111:129–40
324. Deleted in proof
325. Thompson HA, Sadnik I, Scheinbuks J, Moldave K. 1977. *Biochemistry* 16: 2221–30
326. Russell DW, Spremulli LL. 1979. *J. Biol. Chem.* 254:8796–800
327. Valenzuela DM, Chaudhuri A, Maitra U. 1982. *J. Biol. Chem.* 257:7712–19
- 327a. Ceci M, Gaviraghi C, Corrini C, Sala LA, Offenhauser N, et al. 2003. *Nature* 426:579–84
328. Srivastava S, Verschoor A, Frank J. 1992. *J. Mol. Biol.* 226:301–4
329. Hoshino S, Imai M, Kobayashi T, Uchida N, Katada T. 1999. *J. Biol. Chem.* 274:16677–80
330. Cosson B, Couturier A, Chabelskaya S, Kiktev D, Inge-Vechtomov S, et al. 2002. *Mol. Cell Biol.* 22:3301–15
331. Uchida N, Hoshino S, Imataka H, Sonenberg N, Katada T. 2002. *J. Biol. Chem.* 277:50286–92
332. Chou T. 2003. *Biophys. J.* 85:755–73
333. Sonenberg N, Hershey JWB, Mathews MB, eds. 2000. *Translational Control of Gene Expression*. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press
334. Hershey JWB, Mathews MB, Sonenberg N, eds. 1996. *Translational Control*. Cold Spring Harbor, NY: Cold Spring Harbor Lab. Press



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