mRNA Translation: Unexplored Territory in Renal Science

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Ambient protein levels are under coordinated control of transcription, mRNA translation, and degradation. Whereas transcription and degradation mechanisms have been studied in depth in renal science, the role of mRNA translation, the process by which peptide synthesis occurs according to the genetic code that is present in the mRNA, has not received much attention. mRNA translation occurs in three phases: Initiation, elongation, and termination. Each phase is controlled by unique eukaryotic factors. In the initiation phase, mRNA and ribosomal subunits are brought together. During the elongation phase, amino acids are added to the nascent peptide chain in accordance with codon sequences in the mRNA. During the termination phase, the fully synthesized peptide is released from the ribosome for posttranslational processing. Signaling pathways figure prominently in regulation of mRNA translation, particularly the phosphatidylinositol 3 kinase–Akt–mammalian target of rapamycin pathway, the AMP-activated protein kinase–tuberous sclerosis complex protein 1/tuberous sclerosis complex protein 2–Rheb pathway, and the extracellular signal–regulated kinase 1/2 type mitogen-activated protein kinase signaling pathway; there is significant cross-talk among these pathways. Regulation by mRNA translation is suggested when changes in mRNA and protein levels do not correlate and in the setting of rapid protein synthesis. Ongoing work suggests an important role for mRNA translation in compensatory renal growth, hypertrophy and extracellular matrix synthesis in diabetic nephropathy, growth factor synthesis by kidney cells, and glomerulonephritis. Considering that mRNA translation plays an important role in cell growth, development, malignancy, apoptosis, and response to stress, its study should provide novel insights in renal physiology and pathology.

J Am Soc Nephrol 17: 3281–3292, 2006. doi: 10.1681/ASN.2006050488

With the decoding of the human genome, the focus on mechanisms of cell function has understandably shifted to proteins. Although not the sole mediators, proteins are critical for nearly every cell function ranging from mitosis and development to death. There is an urgent need to understand the mechanisms that control protein metabolism and function. The ambient level of a protein is the result of rate of synthesis and rate of degradation. Although synthesis of a protein involves transcription and mRNA translation, until recently, the majority of investigations have addressed transcription. Translation of mRNA, the process by which codon sequences in mRNA are used to synthesize a polypeptide chain, has received scant attention. It is becoming increasingly evident that mRNA levels do not always correlate with the respective protein levels (1). Similarly, a lack of correlation may exist between the level of a protein and its function (2). Comprehensive studies on gene expression, therefore , will need to include posttranscriptional events such as mRNA translation, posttranslational modification such as phosphorylation, and degradation. In this review, we limit our discussion to mechanics of mRNA translation and mechanisms of its regulation, areas that have not been well studied in renal science.

mRNA Translation: Participating Molecules and Cell Structures

mRNA

Eukaryotic mRNA are monocistronic molecules that are produced in the nucleus by the activity of RNA polymerase II. The mRNA molecule contains a "cap" at the 5′ terminus that is made of methylated guanosine triphosphate (m7GpppX where G is guanosine and X is any base; Figure 1A). The stretch of bases from the cap to the first methionine (AUG) codon is not translated and is called the 5′ untranslated region (UTR). The 5′UTR is succeeded by the coding sequence of bases, which in triplets form codons that carry the genetic message for individual amino acids. At its 3′ end, the coding sequence contains a termination codon, which signals ending of peptide chain synthesis. The coding sequence is followed by another stretch of untranslated nucleotides that form the 3′UTR. Several proteins bind to 3′UTR, regulating mRNA stability as well as the initiation phase of mRNA translation. The 3′ terminus of mRNA is made of 20 to 200 adenine nucleotides, the polyA tail, a site that binds proteins (*e.g.*, polyA binding protein [PABP]) that regulate the process of mRNA translation.

Transfer RNA

Accounting for nearly 15% of total cellular RNA, transfer RNA (tRNA) is synthesized in the nucleus under the control of

Published online ahead of print. Publication date available at www.jasn.org.

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ISSN: 1046-6673/1712-3281

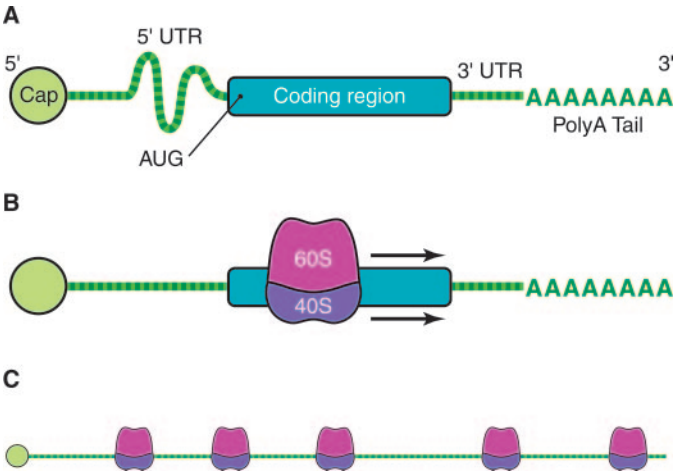

Figure 1. (A) Structure of mRNA. The mRNA carries a "cap" that is made of methylated guanosine triphosphate at the 5′ end. Secondary structures in the 5′ untranslated region (UTR) can impede the ribosome from scanning for the first AUG codon. The coding region carries the sequence of codons for amino acids. (B) Ribosomes. Translation requires the participation of both the 40S and 60S ribosomal subunits, which combine to form the 80S ribosome. The 80S ribosome scans the mRNA coding region in 5′ to 3′ direction (arrows). (C) Polysome. Many 80S ribosomes bind to an mRNA that is targeted for increased translation, forming the polysome. Adapted from reference (117) with permission. Illustration by Josh Gramling—Gramling Medical Illustration.

RNA polymerase III. The tRNA binds to a specific activated amino acid *via* a CCA sequence in the 3′ end of the molecule; this reaction is catalyzed by aminoacyl tRNA synthetase, specific for each amino acid. During the elongation phase, amino acyl tRNA brings the amino acid to the growing peptide chain. A sequence of three bases in the tRNA (anticodon) binds to the codon on the mRNA that is specific for the amino acid being carried. This arrangement permits faithful placement of amino acid in the peptide chain according to the sequence of codons. However, note that the codon on the mRNA does not bind to the amino acid directly; the tRNA functions as an adaptor and brings the amino acid in place by virtue of codon–anticodon alignment.

Ribosomes

Ribosomes are protein-synthesizing structures in the cytoplasm that exist in several forms. They are classified according to sedimentation properties (*e.g.*, 40S, 60S subunits, the 80S unit); the 80S unit is formed by the binding of 40S and 60S subunits. During translation, the mRNA is placed in the groove in the 80S unit that lies between the 40S and 60S subunits (Figure 1B). Cytoplasmic mammalian ribosomes are composed mostly of nucleic acids and proteins; the 40S subunit contains 18S ribosomal RNA (rRNA) and 33 proteins, and the 60S subunit contains 28S, 5.8S, and 5S rRNA and 49 proteins. The 40S subunit is the site where amino acyl tRNA binds to the codon sequence in mRNA; the 60S subunit is involved in formation of peptide bonds between amino acids. rRNA accounts for vast majority of cellular RNA (nearly 80%). Most of the rRNAs are synthesized in the nucleolus under the control of RNA polymerase I. When a particular mRNA is targeted for increased translation, several ribosomes bind to a single molecule and initiate peptide synthesis; such mRNA molecules that carry several ribosomes are called polyribosomes or polysomes (Figure 1C).

Process of mRNA Translation

The process of converting the genetic message in a mRNA into a peptide occurs in three phases: Initiation, elongation, and termination. Each of these phases is controlled intricately by regulatory factors and signaling reactions. An efficient quality control is exerted even as the mRNA is made in the nucleus and transported into cytoplasm to bind with the ribosome and peptide is synthesized.

Initiation Phase

The primary objective of the initiation phase is to bring the 80S ribosomal unit to the mRNA codon (AUG) that corresponds to the first amino acid of the peptide, usually methionine. This can be achieved in at least two ways: Internal ribosomal entry site (IRES)-driven mechanism or cap-dependent scanning mechanism. The latter accounts for translation of the majority of cellular mRNA; however, nearly 10% of cellular mRNA can be translated by the alternative mechanism involving IRES (3).

IRES-Driven Initiation Phase. Persistence of translation of mRNA even when the cap-dependent mechanism is inhibited (4) suggests the presence of alternative pathways. One such mechanism involves binding of the ribosome directly to sequences in mRNA, initially termed ribosome landing pads by Sonenberg *et al.* (5), now called IRES. First described in viruses, many eukaryotic mRNA later were shown to be translated by IRES mechanism, particularly in times of stress, including endoplasmic reticulum (ER) stress, when cap-dependent translation is inhibited (*e.g.*, heat-shock protein 70, Bcl-2, survivin, Ig binding protein) (3). Unlike the cap-dependent pathway of mRNA translation, in the IRES-driven mechanism, ribosomal binding to mRNA does not need eukaryotic initiation factor 4E (eIF4E). Other eIFs, however, may participate in the process (6,7). The precise role of eIF in IRES-driven translation is not known, but eIF4A, a helicase, may unravel complexities in the 5′UTR to facilitate ribosome binding to the mRNA (7). Non-eIF proteins, called IRES-transacting factors, also are involved in regulation of IRES-driven mRNA initiation (*e.g.*, heterogeneous nuclear ribonucleoprotein [hnRNP]) (8). IRES do not share a consensus sequence but contain multiple AUG start sites; their secondary and tertiary structures are thought to facilitate ribosomal binding (3). mRNA of fibroblast growth factor 2 (9), vascular endothelial growth factor (VEGF) (10), ornithine decarboxylase (11), and PDGF (12) can be translated by the IRES-driven mechanism. Some mRNA (*e.g.*, neurogranin [13], VEGF [10,14]) can be translated by both cap-dependent and IRES-driven mechanisms.

Cap-Dependent Mechanism of Initiation. Most eukaryotic mRNAs undergo cap-dependent initiation (4,6). Contro-

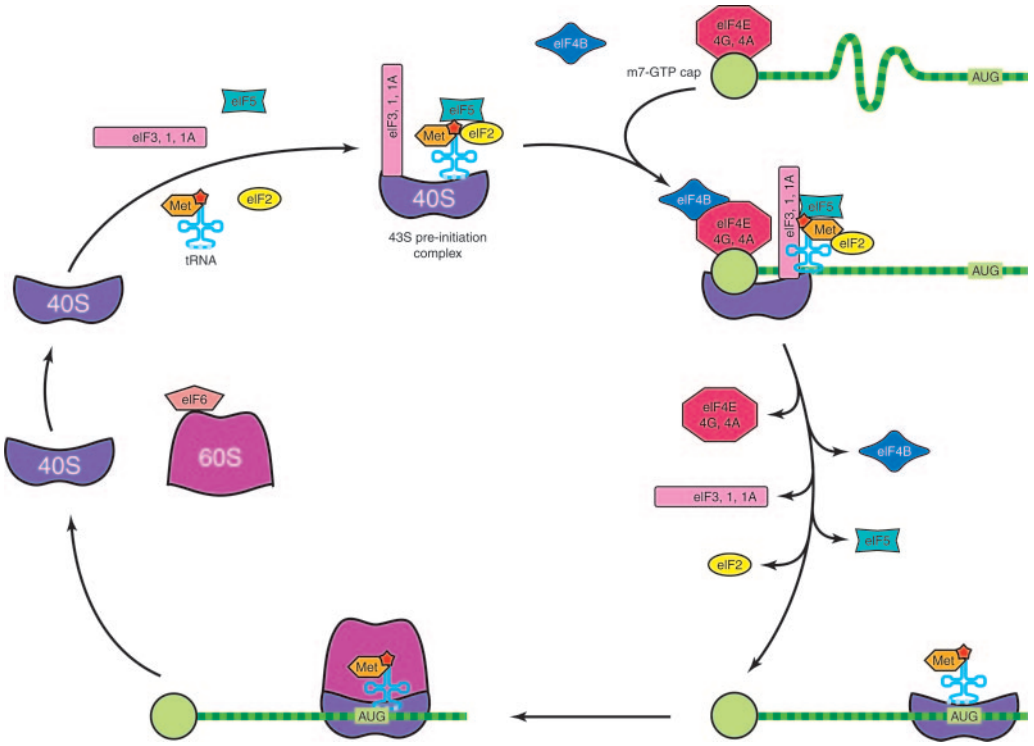

Figure 2. Scanning model of the cap-dependent initiation phase of mRNA translation. The initiation phase begins with formation of the 43S preinitiation complex (40S ribosome unit; eukaryotic initiation factor [eIF] 1, 1A, 2, 3, and 5; and methionine initiator transfer RNA [tRNA]). eIF4F (eIF 4E, 4G, and 4A) binds to mRNA cap. 40S ribosome + methionyl initiator tRNA moves to the first AUG (methionine) codon on mRNA with dissociation of eIF from the ribosome-mRNA complex. The 60S ribosome combines with the 40S unit to form the 80S unit. Adapted from reference (117) with permission. Illustration by Josh Gramling—Gramling Medical Illustration.

versy exists on details of cap-dependent initiation (15), and a consensus scanning model is presented here (Figure 2). At the beginning of the process, the 40S ribosomal subunit forms 43S preinitiation complex with initiator methionyl tRNA (Met-tRNA) bound to eIF2-GTP, eIF1, eIF1A, eIF5, and eIF3 (16). eIF2 consists of three subunits: eIF2α, β, and γ (17). Activation of eIF2α occurs when the associated GDP is exchanged for GTP, facilitated by eIF2B, a guanidine nucleotide exchange factor that binds to eIF2α directly (18). eIF2α plays an important role in regulation of protein synthesis. eIF2α is phosphorylated on Ser51 by double-stranded RNA-activated protein kinase-like ER kinase (PERK), which is activated in states of ER stress and results in inhibition of general protein synthesis (19). At the time of assembly of the 43S ribosomal complex, other eIF—4E, 4A, and 4G—form another complex, called the eIF4F complex, which binds to the cap at the 5′ terminus of the mRNA. In the basal state, eIF4E is held in an inactive complex by one of its three binding proteins (4E-BP1, also called PHAS I). Upon stimulation, 4E-BP1 undergoes phosphorylation and releases eIF4E, allowing it to bind eIF4G (Figure 3). eIF4E is an mRNA cap-binding protein that brings the rest of eIF4F components to proximity of the cap by its association with eIF4G. The binding sites for eIF4G and 4E-BP1 are similar (YXXXXLϕ, where Y is tyrosine, L is leucine, X is any amino acid, and ϕ is a hydrophobic amino acid), and the two proteins compete for eIF4E (20). eIF4G is a large scaffolding protein with distinct binding

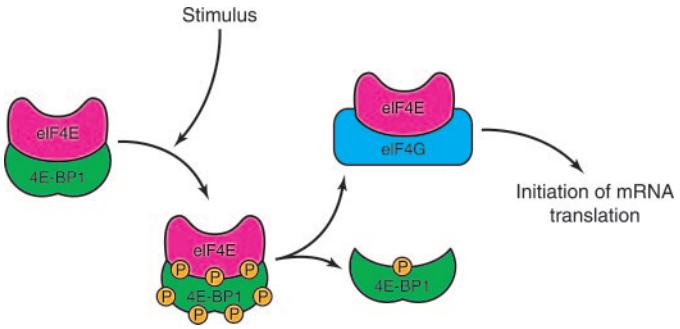

Figure 3. Interaction between eIF4E, 4E-BP1, and eIF4G. In the basal state, eIF4E is held inactive by 4E-BP1. When a stimulus is received for protein synthesis, 4E-BP1 is phosphorylated and dissociates from eIF4E. Free eIF4E binds to eIF4G and facilitates onset of the initiation phase of mRNA translation. Adapted from reference (117) with permission. Illustration by Josh Gramling—Gramling Medical Illustration.

sites for eIF4E, eIF3, and PABP. Although the exact mechanism by which 43S preinitiation complex binds mRNA is not clear, association between eIF3, present in preinitiation complex, and eIF4G, present in the eIF4F complex, may serve this function (4). The scanning ability of the preinitiation complex for the first AUG is impeded when there are secondary structures in the 5′UTR. eIF4A, an RNA helicase, assisted by eIF4B, resolves

these complexities in the 5′UTR and facilitates scanning to locate the AUG by the preinitiation complex. There is disagreement if helicase activity of eIF4A alone, without eIF4B, is sufficient because it may resolve complexities that are only four to five bases long (15). Assembly of preinitiation complex and eIF4F complex at the 5′ terminus and successful scanning for AUG are followed by lodging of Met-tRNA at the AUG codon. There can be several AUG triplets in the 5′UTR; only an AUG codon that is in the right context (*e.g.*, CCA/GCC*AUG*G) can serve as the initiating site for peptide elongation (21). Once the Met-tRNA is positioned on the AUG codon, eIF5 facilitates the hydrolysis of the GTP bound to eIF2 and ultimately the dissociation of initiation factors from the mRNA-ribosomal complex. Upon arrival of Met-tRNA at the AUG codon, the 60S ribosomal subunit combines with the 40S subunit to form the 80S ribosomal unit; at this time, the machinery is in place to commence peptide elongation. This step marks the end of initiation phase of mRNA translation.

In addition to events that occur at the 5′ end of mRNA, the 3′ segment of mRNA is important in regulation of mRNA translation. Factors that interact with 3′UTR include hnRNP, which are involved in chromatin remodeling, transcription, splicing, mRNA stability, and translation. hnRNPK binds to cytidine-uridine (CU)-rich regions of the 3′UTR of several mRNA (22). The role of hnRNPK in regulation of mRNA translation is not fully known. It inhibits translation of 15 lipoxygenase mRNA (23). The polyA tail of mRNA is a binding site for PABP. PABP forms a complex with eIF4G, which is a part of eIF4F complex at the 5′ end of mRNA. This association contributes to mRNA circularization, which is proposed to increase efficiency of mRNA translation (24). PABP also is involved in joining of the 40S and 60S ribosomal subunits during initiation and interaction of eIF4E with the cap structure (25). It should be pointed out that several issues are controversial in the above model (*e.g.*, dephosphorylation of 4E-BP1 is not always accompanied by inactivation of eIF4E [26]). The precise timing of entry and exit of initiation factors also is not known exactly. New initiation factors that play a regulatory role in the initiation phase may still be found.

Elongation Phase

During the elongation phase, peptide propagation occurs by systematic addition of amino acids in accordance with the codon sequence in the mRNA (27–29). The participants in the elongation phase include the mRNA, the amino acyl tRNA, the 80S ribosome made of 40S and 60S subunits, and eukaryotic elongation factors (eEF). The 80S ribosome contains binding sites for tRNA called the aminoacyl (A) site, the peptidyl (P) site, and the exit (E) site (Figure 4). The elongation phase is regulated by eEF. During the initiation phase, the Met-tRNA brings methionine as the first amino acid of the peptide. Once the amino acyl tRNA has been recruited to the 40S subunit, it moves from the A site to the P site on the ribosome, vacating the A site for the arrival of the amino acyl tRNA that bears the next amino acid. Arrival of a new amino acyl tRNA at the P site allows a peptide bond to develop between the current amino acid with the previous one and frees the previous amino acid

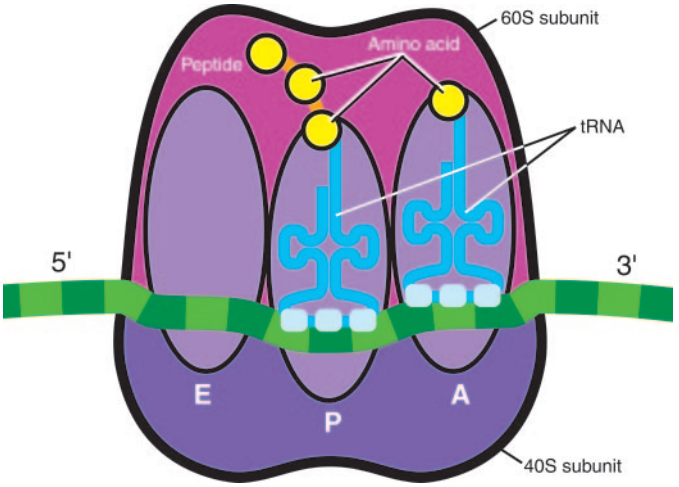

Figure 4. Elongation phase of mRNA translation. mRNA is situated in a groove between the 60S and 40S ribosomal units. Specific amino acyl tRNA bring amino acids, and peptide formation occurs in accordance with codon that are sequences present in the mRNA. Exit (E), peptidyl (P), and amino acyl (A) sites on the ribosomes are shown. Illustration by Josh Gramling—Gramling Medical Illustration.

from its tRNA. The freed tRNA then can be recharged with its cognate amino acid. eEF1A-GTP binds the amino acyl tRNA and places it in alignment with the next codon, a process that requires hydrolysis of GTP. The resulting eEF1A-GDP binds to the eEF1B complex that is made of α, β, and γ isoforms of eEF1B in various combinations. The eEF1B complex facilitates exchange of GDP for GTP on eEF1A. The eEF1A-GTP now is ready to accept the next amino acyl tRNA and bring it to the A site. Next, another factor, eEF2, facilitates the movement of the ribosome in the 5′ to 3′ direction that corresponds to three bases, exactly one codon on the mRNA. This "translocation" process results in movement of the peptide from the A site to the P site; the previous amino acid in the P site moves to the exit (E) site. These events require hydrolysis of GTP bound to eEF2 and for eEF2 to be in its active dephosphorylated state.

Termination Phase

The termination phase begins with the arrival of the 80S ribosome at the stop codon (*e.g.*, UGA) on the mRNA. The release of the peptide is facilitated by the ribosomal release factor, which has structural similarity to tRNA (30). The 80S ribosome is released from the peptide and is split into 40S and 60S subunits, which are recycled for another round of peptide synthesis. Under basal conditions, eIF6 prevents the association of the 60S subunit with the 40S subunit; when a stimulus for protein synthesis is received, eIF6 is phosphorylated and dissociates from the 60S subunit, allowing the formation of 80S ribosome (31).

Signaling Regulation of mRNA Translation

Signaling Regulation of the Initiation Phase. The initiation phase of mRNA translation is rate limiting for protein

synthesis (32). Much of the control of mRNA translation is exerted by signaling reactions, which can occur rapidly.

Phosphatidylinositol 3-Kinase–Akt. In renal proximal tubular epithelial cells, insulin, IGF-I, and VEGF stimulate phosphatidylinositol 3-kinase (PI3-K) activity *via* activation of respective receptor tyrosine kinases (33–37) (Figure 5). Insulin, IGF-I, and VEGF increase tyrosine phosphorylation of insulin receptor substrate-1 and -2 proteins, which bind to p85 regulatory subunit of PI3-K (34–37). Growth factor–induced PI3-K activation, 4E-BP1 phosphorylation, and protein synthesis in renal epithelial cells are dependent on insulin receptor substrate phosphorylation and PI3-K activation (34–37). High glucose also stimulates PI3-K activity in renal epithelial cells (38). These *in vitro* observations correspond to PI3-K activation in the kidney that is undergoing hypertrophy in rodents with type 1 or type 2 diabetes (39) (M.J.L. and B.S.K., unpublished observations). PI3-K activation is required for 4E-BP1 phosphorylation that is induced by insulin (33), IGF-I (34), VEGF (35), and high glucose (38). Actions of PI3-K are opposed by phosphatase and tensin homolog on chromosome ten (PTEN), which dephosphorylates the lipid product of PI3-K; its role in mRNA translation is under investigation. The inositol 3,4,5 trisphosphate lipid product of PI3-K promotes translocation of Akt, a serine-threonine kinase, to the cell membrane. Ser473 on Akt is phosphorylated by mammalian target of rapamycin (mTOR) in association with G protein β subunit–like protein (GβL) and rictor (the TORC2 complex) (40). This is followed by phosphorylation of Thr308 by phosphoinositide-dependent kinase 1 (PDK1) (41). Akt activity is required for 4E-BP1 phosphorylation in response to some stimuli (42), likely because Akt signaling regulates mTOR, the upstream controller of 4E-BP1. In renal epithelial cells, expression of dominant negative Akt abolishes 4E-BP1 phosphorylation and protein synthesis and hypertrophy that is induced by IGF-I and VEGF (34,35). Although Akt initially was reported to promote Ser2448 phosphorylation of mTOR (43), recent investigations have shown that Akt is not a direct kinase for mTOR, and Ser2448 phosphorylation is under the control of p70S6 kinase (44,45).

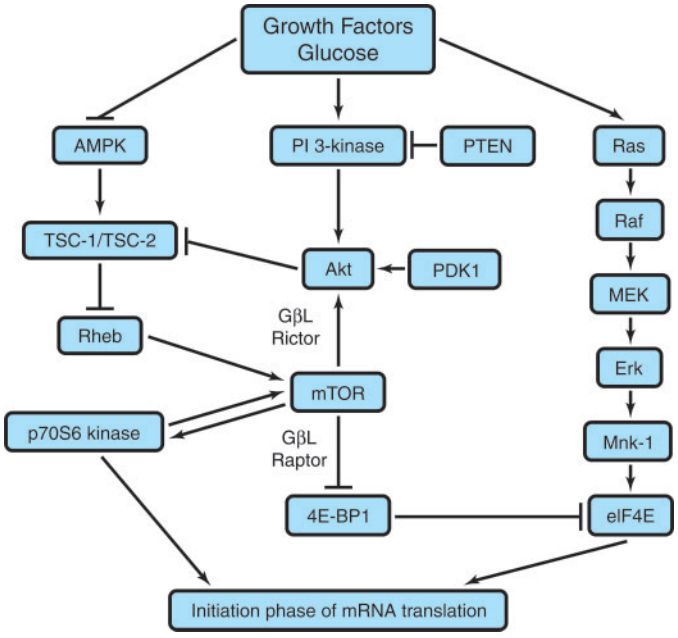

Figure 5. Signaling regulation of phosphorylation of 4E-BP1 and eIF4E leading to stimulation of initiation of mRNA translation. In general, phosphatidylinositol 3-kinase (PI3-K)–Akt–mammalian target of rapamycin (mTOR) pathway regulates phosphorylation of 4E-BP1 and p70S6 kinase, and the Ras–Raf–MEK–extracellular signal–regulated kinase (Erk)–mitogen-activated protein kinase (MAPK)-integrating kinase (Mnk-1) pathway controls phosphorylation of eIF4E during the initiation phase of mRNA translation. AMP-activated protein kinase (AMPK) stimulates tuberous sclerosis complex 1 (TSC-1)/TSC-2, which normally inhibits Rheb and leads to inhibition of mTOR. In states of stimulation of protein synthesis, AMPK activity seems to be reduced, leading to a decrease in activity of TSC-1/TSC-2 and stimulation of Rheb and mTOR activities. Adapted from reference (117) with permission. Illustration by Josh Gramling—Gramling Medical Illustration.

Tuberous Sclerosis Complex Proteins and AMP-Activated Protein Kinase. Synthesis of proteins and mRNA translation consume a significant fraction of cell energy (46). Therefore, it is logical to anticipate that cell energy sensors (*e.g.*, AMP-activated protein kinase [AMPK]) will modulate mRNA translation. AMPK is a trimeric protein with the α subunit containing catalytic activity (Figure 5). Reduction in cellular ATP and increase in AMP content activate AMPK *via* phosphorylation of Thr172 on the α subunit (47), which is accomplished by the kinase LKB-1 (48). AMPK inhibits energy-consuming processes such as protein synthesis and stimulates ATP-generating reactions such as fatty acid oxidation. AMPK phosphorylates tuberous sclerosis complex 2 (TSC-2), and this is thought to increase its activity (46). TSC-2 (tuberin) and TSC-1 (hamartin) are gene products of *tsc* genes, which, when mutated, lead to cell hypertrophy and hamartomas in the kidney (49), suggesting that they normally inhibit protein synthesis and cell growth (50,51). TSC-1 and TSC-2 form a heterodimer that decreases mTOR activity (52) *via* inhibition of Rheb (ras homolog enriched in brain). Rheb bound to GTP directly binds mTOR and stimulates its activity (53). TSC-2 acts as a GTPase-activating protein and inactivates Rheb (46,53). Akt phosphorylates TSC-2, inactivates it, and leads to mTOR activation (54,55). Akt also phosphorylates AMPK, leading to its inactivation and removal of inhibition on downstream mTOR activity (56). Thus, mTOR activation by Akt seems to be indirect *via* regulation of TSC-1/-2 and AMPK (45). Extracellular signal–regulated kinase (Erk)-type mitogen-activated protein kinase (MAPK) and protein kinase C also phosphorylate TSC-2 (57,58).

mTOR. Activation of mTOR occupies a central role in the regulation of the initiation and elongation phases of mRNA translation. It is a large, modular protein that belongs to the PI3-K–related kinase family of proteins. Function of mTOR depends on the formation of two complexes that differ in their sensitivity to rapamycin. mTOR complex 1 (TORC1) is rapamycin sensitive and consists of mTOR, GβL (also called LST8), and regulatory-associated protein of TOR (raptor). GβL is required for association between raptor and mTOR (59). Raptor facilitates mTOR binding to TOR signaling motifs (TOS do-

mains) in its targets 4E-BP1 and p70S6 kinase (60,61). mTOR serves as a nutrient sensor, and TORC1 activity is increased when nutrient supply is abundant. Nutrients may regulate TORC1 *via* regulation of the AMPK–TSC-2–Rheb pathway (62). In the context of stimulation of protein synthesis in proximal tubular epithelial cells, insulin, IGF-I, angiotensin II (AngII), VEGF, and high glucose recruit the PI3-K–Akt pathway to promote TORC1 activity as indicated by an increase in phosphorylation of its direct substrate, 4E-BP1 (Figure 5) (33–37). These data support a role for TORC1 in diabetes-induced renal hypertrophy. Cellular stresses such as hypoxia and DNA damage inhibit TORC1 activity and suppress protein synthesis. Hypoxia promotes activation of REDD1, a protein that may be upstream of TSC-1/TSC-2 (63), and leads to inhibition of TORC1. TORC1 also regulates cell proliferation; in an animal model of polycystic kidney disease, mTOR inhibition with rapamycin led to a decrease in mitotic activity of epithelial cells that lined the cysts and those that lined the noncystic tubules and reduction in cyst volume density (64).

Stimulation of protein synthesis by TORC1 involves activation of factors that regulate the initiation and elongation phases of mRNA translation and ribosomal protein phosphorylation and biogenesis. Phosphorylation of 4E-BP1 on Thr37,46 is under the control of mTOR (65). Phosphorylation of p70S6 kinase, another direct substrate of TORC1, leads to phosphorylation of the 40S ribosomal protein S6 (6). The earlier notion that p70S6 kinase may participate in translation of mRNA that contain the 5′ tract of oligopyrimidine (5′TOP) that code for ribosomal proteins and elongation factors (66) has been disproved (67,68). eIF3, a component of the 43S preinitiation complex, associates with p70S6 kinase in the resting cell. Upon stimulation of protein synthesis, activated TORC1 phosphorylates p70S6 kinase and displaces it from eIF3, allowing TORC1 to bind eIF3 (32). Therefore, mTOR can influence formation of 43S preinitiation complex.

TORC2, made of mTOR, GβL, and rictor and, possibly, other proteins is resistant to rapamycin. TORC2 phosphorylates Ser473 of Akt (40) and also regulates actin organization, leading to changes in cell shape (69). Upstream regulators and downstream targets of TORC2 need to be explored in depth.

Regulation of 4E-BP1 Phosphorylation. There are at least seven threonine or serine phosphorylation sites on 4E-BP1 that undergo phosphorylation (70). Akt and mTOR-dependent phosphorylation of Thr37,46 may be the priming event (Figure 5) (42) that leads to phosphorylation of Thr70 and Ser65; phosphorylation at these four sites may be enough to release eIF4E (70). Some suggest that phosphorylation at Ser65 and Ser111 may not be needed for release of eIF4E (71). Other kinases, such as cdc 2 and ataxia telangiectasia mutated, may act as kinases for 4E-BP1 (72,73). Insulin-induced 4E-BP1 phosphorylation depends on Erk activation in renal proximal tubular epithelial cells (33).

Regulation of eIF4E Phosphorylation by the Erk–MAPK-Integrating Kinase System. Ser209 on eIF4E undergoes phosphorylation (74), a process that is Erk 1/2-type MAPK dependent. Erk is not the direct kinase but activates MAPK-integrating kinase (Mnk-1), which directly phosphorylates Ser209 on eIF4E (75). This is facilitated by eIF4G, which has binding sites for both eIF4E and Mnk-1 (75). The role of phosphorylation of eIF4E in regulation of mRNA translation is not fully understood (76). However, in proximal tubular epithelial cells, Erk-dependent induction of Mnk-1 phosphorylation is needed for eIF4E phosphorylation and stimulation of protein synthesis by VEGF (77).

Signaling Regulation of the Elongation Phase. eEF1 level is controlled by transcription, and its amount is increased in skeletal muscle in diabetes (78). eEF2 is a GTP-binding protein that is inhibited by phosphorylation of Thr56 by eEF2 kinase (79). Stimulation of protein synthesis by insulin is associated with dephosphorylation of Thr56 that is mTOR dependent (80) (Figure 6). In some cells, dephosphorylation of eEF2 may be regulated by the Erk-type MAPK–p90 rsk axis (81,82). These kinases regulate the activity of eEF2 kinase to modulate Thr56 phosphorylation of eEF2. Insulin- and high glucose–induced stimulation of protein synthesis is associated with reduction in eEF2 phosphorylation on Thr56 in proximal tubular epithelial and glomerular epithelial cells (GEC; M.M.M., M.J.L., B.S.K., unpublished observations). Phosphorylation of eEF2 is catalyzed by the highly specific eEF2 kinase, a calcium-calmodulin–dependent enzyme (83). Reduction in eEF2 kinase activity, seen when protein synthesis is stimulated, involves phosphorylation of sites that inactivate the enzyme (*e.g.*, Ser366 and Ser78) (84). Ser366 phosphorylation is under the control of p70S6 kinase and p90rsk (82,84). AMPK increases eEF2 kinase activity by

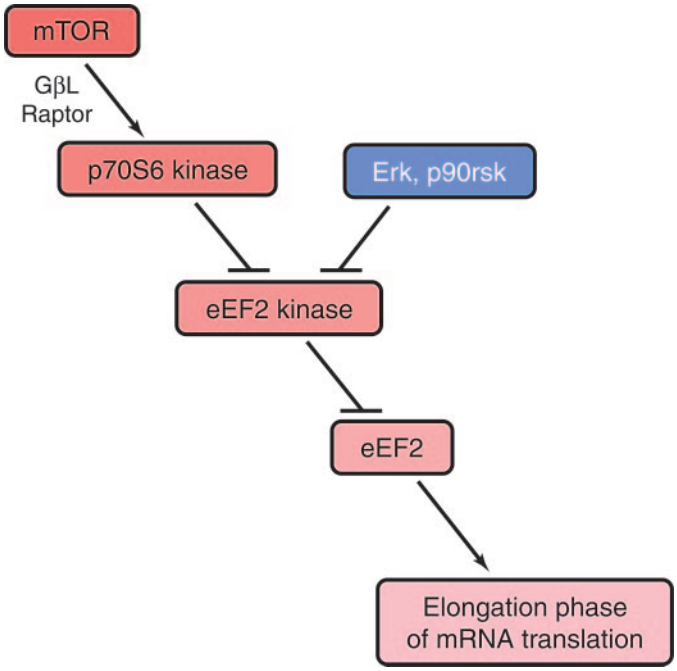

Figure 6. Signaling regulation of eEF2 phosphorylation leading to stimulation of the elongation phase of mRNA translation. p70S6 kinase, Erk, and p90rsk increase phosphorylation of eEF2 kinase, leading to its inactivation and resulting in inhibition of generation of phosphorylated eEF2. Phosphatases such as PP2A also can increase the content of dephosphorylated eEF2. The elongation phase is facilitated by dephosphorylated eEF2. Illustration by Josh Gramling—Gramling Medical Illustration.

phosphorylation of Ser398 and inhibits the elongation phase of protein synthesis, when most of the energy is consumed (85). Phosphatases (*e.g.*, PP2A) also regulate dephosphorylation of eEF2 under the control of mTOR and α4 protein (86).

mRNA Translation in Renal Physiology and Pathology

Compensatory Hypertrophy

After removal of a kidney, the contralateral kidney undergoes adaptive growth that is due mostly to increase in cell size from augmented content of RNA and protein content (*i.e.*, hypertrophy) (87). Renal hypertrophy is linked closely to cell-cycle events (88,89). Increase in cell protein, attained through stimulation of protein synthesis, is likely to involve altered regulation of mRNA translation. Chen *et al.* (90) studied the regulation of mRNA translation in compensatory growth after uninephrectomy. In the hypertrophic renal parenchyma, an increase in phosphorylation of ribosomal protein S6 and 4E-BP1 phosphorylation could be seen as early as 30 min and 6 h, respectively, after uninephrectomy, demonstrating activation of mTOR. Thr37,46,70 and Ser65 were identified as sites of phosphorylation of 4E-BP1. Phosphorylation of both ribosomal protein S6 and 4E-BP1 was abolished by administration of rapamycin, an mTOR inhibitor, which also significantly inhibited the extent of kidney growth at 1 and 4 d. This important study demonstrated that mTOR activation is a central event in compensatory renal growth and identified it as a potential therapeutic target in pathologic states in which kidney hypertrophy may predispose to chronic kidney pathology.

Diabetic Nephropathy

Important events in the evolution of diabetic nephropathy include hypertrophy and accumulation of extracellular matrix (ECM). Because both these processes are contingent on increased protein synthesis, we investigated the role of mRNA translation.

Diabetes-Induced Renal Hypertrophy. The first structural change in the kidney in both type 1 and type 2 diabetes is hypertrophy, which occurs rapidly (35). The importance of hypertrophy lies in the possibility that it predisposes to long-term renal complications of diabetes (91,92). Because hyperglycemia alters the energy status of the cell and AMPK is an energy sensor, we hypothesized that AMPK regulates diabetes-induced renal hypertrophy and high-glucose–induced hypertrophy in GEC. Understanding the mechanisms of injury to GEC is important because decrease in their number and/or density may contribute to proteinuria and progression of diabetic renal disease (93–95). In the GEC, high glucose stimulated *de novo* protein synthesis and induced hypertrophy (96), in association with increased Thr37/46 phosphorylation of 4E-BP1 and decreased Thr56 phosphorylation of eEF2, suggesting stimulation of both the initiation and elongation phases of mRNA translation. High glucose–stimulated protein synthesis depended on PI3-K, Akt, and mTOR. High glucose reduced AMPKα subunit Thr172 phosphorylation in an Akt-dependent manner. Agents that increase AMPK activity, metformin and 5-aminoimidazole-4-carboxamide-1-β-riboside (AICAR), inhibited high-glucose stimulation of protein synthesis.

Renal hypertrophy in rats with type 1 diabetes was associated with a reduction in AMPK phosphorylation and an increase in mTOR activity. In diabetic rats, metformin and AICAR increased renal AMPK phosphorylation, reversed mTOR activation, and inhibited renal hypertrophy, without affecting hyperglycemia. These data suggest that in the basal state, AMPK is an inhibitor of protein synthesis; high glucose reduces AMPK activity by the PI3-K–Akt pathway, thereby facilitating mTOR activity (Figure 5). These data suggest that AMPK may be a potential therapeutic target in diabetes-related kidney disease. Glomerular hypertrophy in rats with streptozotocin-induced diabetes was associated with increased expression of growth arrest–specific gene 6 (Gas6), a growth factor, and Axl, its cognate receptor (97). Studies in Gas6-deficient mice that were given streptozotocin showed that the Gas6-Axl system was required for diabetes-induced renal hypertrophy. High-glucose incubation of mesangial cells from control mice resulted in increased expression of Gas6 mRNA and Axl protein and stimulation of Akt phosphorylation and activity of mTOR, the latter indicated by increased phosphorylation of p70S6 kinase and 4E-BP1. However, these changes were not seen in mesangial cells from Gas6 knockout mice, providing evidence for induction of mRNA translation by the Gas6-Axl pathway (98). Gas6-mediated mesangial cell hypertrophy seems to involve PI3-K–Akt–mTOR pathway but not the Erk pathway.

Diabetes-Induced Matrix Synthesis. Progressive loss of renal function in diabetes correlates with accumulation of ECM. Laminin, a trimeric protein constituent of renal ECM, has distinct composition in glomerular and tubulointerstitial compartments (99). Unlike type IV collagen, laminin accumulation in the kidney in db/db mice with type 2 diabetes is associated with reduction in mRNA of its chains α5, β1, and γ1 (100), suggesting that decreased degradation and/or augmented mRNA translation may be involved. We examined regulation of renal laminin β1 mRNA translation in the early phase of type 2 diabetes in db/db mice (K.S. and B.S.K., unpublished observations). In the renal cortex of db/db mice, activity of PI3-K and Akt (39) and mTOR activity was increased. Increment in Thr37,46 phosphorylation of 4E-BP1 and reduction in eEF2 Thr56 phosphorylation indicated activation of the initiation and elongation phases of translation in diabetic renal cortex. Because db/db mice manifest both hyperglycemia and hyperinsulinemia at this early stage, the role of high glucose, high insulin, and both together was explored in proximal tubular epithelial cells (38). The three conditions increased laminin β1 chain protein synthesis within 5 min, lasting for up to 60 min with no change in laminin β1 mRNA levels. Induction of laminin β1 chain synthesis depended on PI3-K, Akt, and mTOR; in addition Erk activation was found to be required. Stimulation of laminin synthesis by insulin suggests a role for insulin in pathogenesis of diabetic kidney disease. Insulin receptor signaling is increased in the renal cortex of db/db mice in contrast to liver tissue from the same animals, demonstrating that kidney is sensitive to actions of insulin in type 2 diabetes in

contrast to liver (39). Hyperinsulinemia also has been implicated in vascular wall and eye pathology in type 2 diabetes (101,102). Rapid induction of laminin mRNA translation by high glucose and high insulin may be of pathophysiologic significance because recent reports show a significant correlation between cardiovascular morbidity and mortality with short-term peaks of high glucose in the postprandial state (103). Therefore, mRNA translation may be a contributing mechanism for matrix accumulation in the diabetic kidney.

TGF-β is an important mediator of renal hypertrophy and matrix expansion in diabetes (104). High glucose augments TGF-β mRNA in proximal tubular epithelial cells; successful translation of TGF-β mRNA into protein, however, depends on PDGF (105). Recently, Lloberas *et al.* (106) reported that administration of rapamycin to rats with streptozotocin-induced type 1 diabetes resulted in reduction in TGF-β mRNA expression, mesangial matrix expansion, and albuminuria but not glomerular hypertrophy.

Angiotensin II (AngII)–Mediated Renal Injury. AngII contributes to chronic progressive renal injury by its hemodynamic and cell biologic effects (107). The latter effects may be mediated both by direct means and *via* activation of growth factors such as TGF-β. VEGF expression is increased in renal cortex in association with renal hypertrophy in mice with type 1 or type 2 diabetes, suggesting a role for VEGF in diabetic renal hypertrophy (35). Neutralizing antibodies ameliorate renal disease in rodents with type 1 or type 2 diabetes, showing that VEGF has a pathogenic role (108,109). We examined potential regulation of VEGF synthesis by AngII in proximal tubular epithelial cells and in renal cortex of mice with type 1 diabetes. AngII (1 nM) stimulated within minutes rapid synthesis of VEGF that depended on mRNA translation and not transcription (14). Polysomal assay revealed enrichment of polysomes with VEGF mRNA showing stimulation of initiation of VEGF mRNA translation, which required 4E-BP1 phosphorylation that was induced by PI3-K, Akt, and mTOR (14). Rapid induction of VEGF translation by AngII required reactive oxygen species that originated from the NAD(P)H oxidase system rather than the mitochondrial respiratory chain (110). The role of the 3′UTR of VEGF mRNA in regulation of VEGF mRNA translation was explored. AngII stimulation of VEGF mRNA translation partly depended on increased binding of hnRNPK to the 3′ end of VEGF mRNA (111). Activity of hnRNPK depended on its phosphorylation on Ser302, which was mediated by protein kinase Cδ. AngII also promoted formation of a multimeric complex, including hnRNPK and eIF4G. Because hnRNPK binds to the 3′UTR, whereas eIF4G binds to the 5′ end of the mRNA, their association may promote circularization of VEGF mRNA. mRNA circularization has been found to increase efficiency of its translation (112).

ER Stress in Renal Disease. ER stress is seen after accumulation of misfolded proteins in the ER, after exposure to inhibitors of protein synthesis, and in metabolic syndrome. In ER stress, general protein synthesis is inhibited by eIF2α phosphorylation by activation of PERK, a kinase; however, synthesis of select proteins is stimulated. Apoptosis also may occur (113). Recent reports indicate that pathogenesis of Heymann nephritis, a model of membranous glomerulonephritis, includes ER stress (114). In isolated glomeruli from rats with passive Heymann nephritis and in GEC that were exposed to membrane attack complex, PERK was activated, leading to an increase in eIF2α phosphorylation. As reviewed previously, phosphorylation of eIF2α by PERK on Ser51 inhibits assembly of the preinitiation complex that contains Met-tRNA, resulting in inhibition of general protein synthesis (19). In cultured cells, eIF2α phosphorylation was associated with a decrease in general protein synthesis; however, synthesis of nephrin, a protein that contributes to permselectivity barrier function of glomerulus, was increased (114). ER stress–associated caspase 12 activation has been implicated in cis-platinum–induced apoptosis in LLC-PK1 cells with implications for acute tubular necrosis (115). Overexpression of megsin, a serine protease inhibitor, in the rat is associated with ER stress as a result of accumulation of unfolded proteins in the ER and is associated with podocyte injury and proteinuria (116).

Conclusions and Future Prospects

Proteins are executors of cell function. The initiation phase of mRNA translation is the rate-limiting step in gene expression, culminating in protein synthesis. It is important not to rely on mRNA level as an index of change in protein level because the two frequently do not correlate; proteins should be studied directly. Regulation at the level of mRNA translation as an independent mechanism is suggested when mRNA and protein levels do not correlate and when proteins are synthesized rapidly. In view of the rapidity with which translation can be stimulated, it is important to investigate changes in synthesis of proteins within minutes of agonist application; otherwise, the early phase of regulation may be missed. Conditions with altered energy states are likely to regulate mRNA translation because the latter consumes a significant part of cell energy. Therefore, translation is a relevant area for investigation in renal diseases with altered energy metabolism, such as acute renal failure and diabetes. Most mRNA translation regulation occurs *via* signaling reactions, allowing rapid responses to occur. Recent investigations have revealed mRNA translation to be an important site of regulation in synthesis of growth factors and matrix proteins in the kidney relevant to compensatory renal growth, diabetic nephropathy, and glomerulonephritis. Because mRNA translation is important as a site of regulation of such proteins as ornithine decarboxylase, VEGF, TGF-β, and laminin, it is likely to be a fruitful area of investigation in mitosis, development, differentiation, malignancy, apoptosis, and ER stress in the kidney. Inhibition of protein synthesis also may involve interruption in translation and as such could merit investigation in renal disease related to ER stress and toxic nephropathies, such as puromycin aminonucleoside nephrosis. Future investigation is very likely to identify proteins that are important in renal function and disease that undergo regulation by mRNA translation. One simple guideline is to evaluate mRNA translation as a mechanism when synthesis of a protein is regulated rapidly and is dissociated from corresponding changes in its mRNA. Regulation of a protein at the levels of transcription and degradation cannot exclude additional con-

trol at the level of translation; however, assessment of relative contribution of each of these mechanisms will require sophisticated mathematical analysis.

Acknowledgments

Work contained in this article was supported by the National Institutes of Health, VA merit review program, American Diabetes Association, Juvenile Diabetes Foundation International, and the National Kidney Foundation of South and Central Texas.

We thank Drs. Christopher Proud and G. Ghosh Choudhury for helpful suggestions.

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