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Review

Regulation of mRNA translation in renal physiology and disease

Balakuntalam S. Kasinath, Denis Feliers, Kavithalakshmi Sataranatarajan, Goutam Ghosh Choudhury, Myung Ja Lee, and Meenalakshmi M. Mariappan

Division of Nephrology, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas

Kasinath BS, Feliers D, Sataranatarajan K, Ghosh Choudhury G, Lee MJ, Mariappan MM. Regulation of mRNA translation in renal physiology and disease. *Am J Physiol Renal Physiol* 297: F1153–F1165, 2009. First published June 17, 2009; doi:10.1152/ajprenal.90748.2008.—Translation, a process of generating a peptide from the codons present in messenger RNA, can be a site of independent regulation of protein synthesis; it has not been well studied in the kidney. Translation occurs in three stages (initiation, elongation, and termination), each with its own set of regulatory factors. Mechanisms controlling translation include small inhibitory RNAs such as microRNAs, binding proteins, and signaling reactions. Role of translation in renal injury in diabetes, endoplasmic reticulum stress, acute kidney injury, and, in physiological adaptation to loss of nephrons is reviewed here. Contribution of mRNA translation to physiology and disease is not well understood. Because it is involved in such diverse areas as development and cancer, it should prove a fertile field for investigation in renal science.

protein synthesis; signaling regulation; diabetic nephropathy; compensatory renal hypertrophy; endoplasmic reticulum stress

RIBONUCLEIC ACID (RNA) is a critical component of the process of gene expression. Some argue life may have begun as RNA, since it is able to replicate, store information, and catalyze some of the fundamental reactions involved in gene expression. An important function of the RNA is to serve as an intermediary (messenger RNA, mRNA) between the genetic notion of a protein and its actual production. It is more. It is a structural component of protein manufacturing ribosomes (ribosomal RNA, rRNA). In the form of transfer RNA (tRNA) it can convey amino acids to the ribosome for peptide synthesis. Noncoding parts of DNA generate RNA species that have important regulatory control over gene expression, e.g., microRNA.

Until recently, a linear stoichiometric relationship between the amount of RNA and its corresponding protein was assumed to exist; however, this is rapidly changing. A consensus is emerging that mRNA levels and protein levels do not always correspond and that proteins deserve direct examination (16, 50). Ambient levels of a protein are the product of interaction between synthesis and degradation; thus, changes in rate of breakdown of a protein can contribute importantly to the nonlinear relationship between mRNA and protein levels. A comprehensive investigation of proteins requires studies on transcription, translation, degradation, posttranslational modification, and function (77, 84). Disturbances in each of these aspects of protein metabolism can affect physiological function of proteins and result in disease.

The first step in protein synthesis is the generation of mRNA from the complimentary DNA (cDNA) sequence by the process of transcription. The process by which the genetic message present in the mRNA is utilized by ribosomes to generate a polypeptide chain with the help of tRNA is called mRNA translation. The newly synthesized peptide may undergo posttranslational modification such as glycosylation, acetylation, and phosphorylation. Having served its function in specific domains of the cell, the protein undergoes degradation. More recently, the importance of translation as an independent regulator step in synthesis of a protein is being recognized (63, 136). mRNA translation plays an important role in control of development, differentiation, tumorigenesis, inflammation, apoptosis, and cell survival (113). In this review, we will focus on recent developments in regulation of mRNA translation with specific reference to kidney physiology and pathology.

RNA

Types of RNA. There are many species of RNA. Just 2% of the human genome codes for mRNA. Large portions of noncoding DNA were thought to represent "junk." Now it is known that nearly 60–70% of the genome codes for noncoding RNAs such as rRNA, tRNA, small nuclear RNA, small nucleolar RNA, microRNA (miRNA), vault RNAs, Y RNAs, rasiRNAs, and, piwi-interacting RNAs (piRNAs) (5). Abnormalities in noncoding DNA can lead to disease, e.g., microsatellite CTG repeats in the 3′-untranslated region of *DMPK* gene in myotonic dystrophy type 1 (109).

Transport of mRNA and cellular localization. After its production in the nucleus, mRNA is transported across the nuclear membrane being bound to a number of proteins, the complex being called the messenger ribonucleoprotein particles (mRNPs). Efficient transport of mRNPs across the nuclear pore requires participation of Dbp5, a member of the DEAD-box protein family, Gle1 protein, and inositol polyphosphate6 (InsP6). The ATPase activity of Dbp5, augmented by its association with Gle1 and InsP6, appears to occur on the cytoplasmic side of the nuclear pore complex and is thought to

Address for reprint requests and other correspondence: B. S. Kasinath, Division of Nephrology, Dept. of Medicine, Univ. of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900 (e-mail: kasinath@uthscsa.edu).

release proteins associated with the mRNA at the nuclear pore so that they can be recycled for use of subsequent mRNA transport (22). The spatial distribution of mRNA following its appearance in the cytoplasm is also tightly regulated and depends on whether the protein product is membrane bound, meant for secretion or, destined for cytoplasmic localization. It used to be thought that secreted proteins with leader sequences were first translated and then transported to the proper cell domain for secretion. Recent investigations have shown that mRNAs may be transported to the final destination and translated locally in the cell. In the *Drosophila* embryo, a large proportion of the mRNAs was found to undergo such subcellular localization (85). There are defined control systems regulating localized translation in subcellular sites (6).

Domains of mRNA. Specific domains in the mRNA play unique functional roles in translation (Fig. 1). A "cap" made of methylated guanidine-P-P-P is located at the 5′-end. The sequence of bases from the cap to the first codon (usually AUG) is the 5′-untranslated region (5′-UTR); complexities in 5′-UTR can impede ribosomal scanning for the AUG during translation. The region with triplet codons carrying cues for specific amino acids (coding sequence) is located downstream of 5′-UTR. This is followed by the 3′-UTR, a site of regulation of mRNA stability and translation efficiency. Following 3′-UTR, several adenosine bases form the poly(A) tail that binds proteins, e.g., poly(A)-binding protein (PABP) (75), and is involved in mRNA stability (132). Electron microscopy studies have shown that mRNA is, in fact, circular due to interactions of the binding proteins at the 5′- and 3′-ends. This may have functional significance as discussed later. Translation involves other RNA species. The aminoacyl-tRNAs transport specific amino acids to the nascent polypeptide. rRNAs are important constituents of ribosomes: the 40S subunit made of 18S rRNA and 33 proteins, and, the 60S subunit made of 28S, 5.8S, and 5S rRNA and 49 proteins (76, 77). During translation, the two ribosomal subunits unite to form the 80S unit.

Process of mRNA Translation

Eukaryotic translation occurs in the following three stages: initiation stage, elongation stage, and termination stage. Much of the control of translation occurs in the initiation phase (139). The reader is referred to recent publications for more a detailed description of the process of translation (76, 77, 113, 139). Movies are available online to aid in understanding the dynamic process of translation (http://www.molecularmovies.com/showcase/).

Initiation stage. Most of the eukaryotic mRNAs are translated by the scanning mechanism. Some mRNAs are translated by the internal ribosome entry (IRES) mechanism. There are controversies regarding the steps and regulation of translation (84).

Scanning model. At first, two multimeric complexes are formed by the participation of eukaryotic initiation factors (eIFs) and the 40S ribosomal subunit. The preinitiation complex is formed by eIF1, eIF1A, eIF2.GTP + initiator methionyl-tRNA, eIF3, eIF5, and the 40S ribosomal subunit. eIF4F complex is constituted by binding of eIF4G, eIF4E, and eIF4A. eIF4E binds to the cap of the mRNA and eIF4G. In addition to eIF4E and eIF4A, eIF4G, a scaffolding protein, also binds eIF3 in the preinitiation complex (81). These associations result in bringing the two multimeric complexes together with mRNA. The 40S ribosomal subunit attempts to scan for the first AUG codon on the mRNA. Any complexities in the 5′-UTR that impede ribosomal scanning are resolved by the helicase activity of eIF4A, probably assisted by eIF4B. Arrival of the 40S ribosomal subunit with initiator methionyl tRNA at the translation competent first AUG codon results in dissociation of eIF1. This is followed by addition of the 60S ribosomal subunit to the mRNA-bound 40S ribosomal subunit to form the 80S ribosomal unit and release of other eIFs. This marks the end of the initiation stage (Fig. 2).

IRES model. Unlike the scanning model, the IRES mechanism of initiation does not need participation of eIF4E although other eIFs do take part. Here, 40S ribosomal subunit binds to certain parts of the 5′-UTR known as IRES (111). The specific features of these ribosomal entry sites are not well understood, but their secondary structures are believed to play an important role. Recently, IRES-binding proteins have been identified that regulate translation (140). Of interest to the kidney, translation of vascular endothelial growth factor (VEGF) and ornithine decarboxylase mRNAs sometimes occurs by this mechanism. IRES-based translation is of importance during cell stress when general cap-dependent translation is inhibited.

Elongation stage. The objective of translation, i.e., synthesizing the peptide, is achieved during the elongation stage. The first step in the elongation stage is the arrival of the amino acyl-tRNA bearing a specific amino acid corresponding to the codon on the mRNA. The recruitment of amino acyl-tRNA to the A (aminoacyl) site on the 80S ribosomal unit is facilitated by GTP-bound eukaryotic elongation factor 1A (eEF1A.GTP). This is followed by shift of the tRNA to the peptidyl (P) site on the ribosome. The movement of amino acyl-tRNA from the A site to the P site is helped by the activity of eEF2.GTP, which is active when dephosphorylated on Thr^{56}. Dephosphorylated eEF2 is believed to move the ribosomal complex exactly three bases such that amino acyl-tRNA in effect moves from the A site to the P site (76, 113). At the P site, a peptide bond is

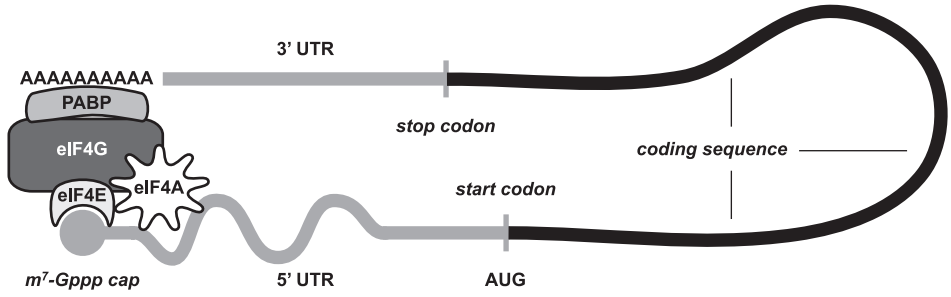

Fig. 1. Functional domains of messenger RNA (mRNA). The 5′- end of the mRNA contains the guanosine-P-P-P as the cap. This is followed by the 5′-untranslated region (UTR), the coding sequence interposed between the start and stop codons, 3′-UTR, and the poly(A) tail. Eukaryotic initiation factor (eIF) 4E forms a multimeric complex with eIF4G and eIF4A and binds to the cap of the mRNA. Poly(A)-binding protein (PABP) binds to the poly(A) tail and to eIF4G, rendering the mRNA circular.

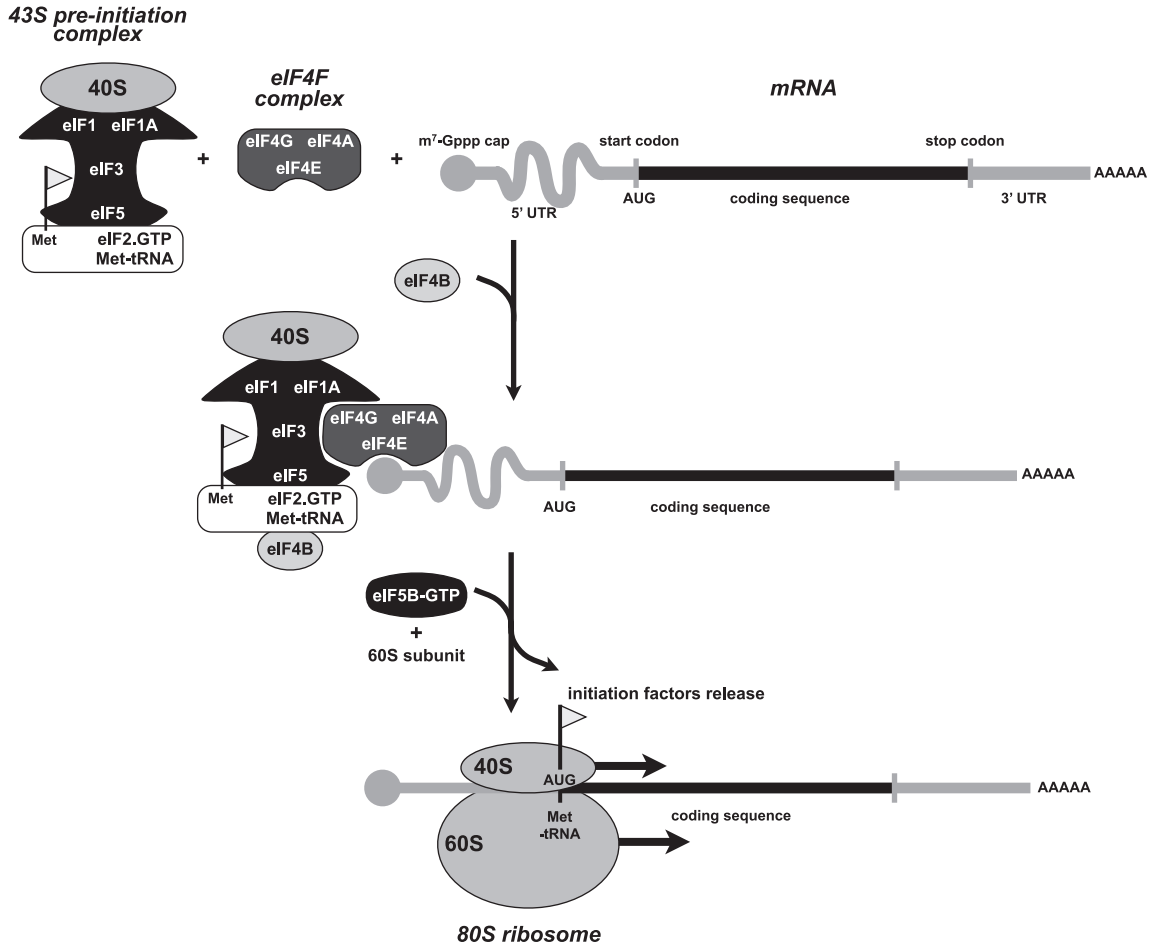

Fig. 2. Scanning model of initiation stage of mRNA translation. A linearized form of mRNA is shown to simplify the figure.

created between the previous amino acid and the one that has arrived newly, and this is accompanied by release of tRNA from the previous amino acid. The released tRNA can then be reused to deliver the specific amino acid as dictated by the codon sequence on the mRNA.

Termination stage. The synthesis of the peptide ceases when the ribosomal complex encounters the termination codon on the mRNA. Ribosomal release factor assists in the process (59). At the end of peptide synthesis, 80S ribosomal complex dissociates into 40S and 60S subunits, a process assisted by eIF6 (14). The ribosomal subunits can be reused for the next cycle of peptide synthesis.

Regulation of Translation

Considering the importance of protein synthesis in cell function, it is not surprising that several different modes of regulation exist in controlling the process of translation. They include posttranslational modifications of regulatory proteins, such as phosphorylation and association with GTP, changes in amounts of regulatory proteins (e.g., eEF1), presence or absence of binding proteins (e.g., 4E-BPs, hnRNPs), and presence or absence of binding RNAs (microRNAs).

Posttranslational modifications. PHOSPHORYLATION. One of the features of mRNA translation is the rapidity with which it occurs following stimulus application. For example, incubation of proximal tubular epithelial cells with ANG II results in synthesis of VEGF within 5 min, a process shown to be due to augmented efficiency of translation of VEGF mRNA (33). It is logical to anticipate that the regulatory reactions will be rapid as well. This is made possible by phosphorylation reactions by specific kinases that can be activated in a matter of seconds. Activity of regulatory factors can be affected in a positive or negative direction by phosphorylation. For example, phosphorylation of 4E-BP1 dissociates it from eIF4E, allowing eIF4E to bind the cap of the mRNA (87, 110); activity of eEF2 during the elongation phase depends on dephosphorylation on Thr^{56} (115).

ASSOCIATION WITH GTP. In addition to phosphorylation, association with GTP can also activate initiation and elongation factors. Thus activities of eIF2α, eIF5, eEF1A, eEF1, and eEF2 are augmented by association with GTP and inhibited by association with GDP. There are distinct guanidine nucleotide exchange factors that dictate these reactions.

Other binding proteins. Activity of some of the initiation factors in translation is controlled by their binding proteins. The association between eIF4E and its binding proteins, 4E-BPs, is a prime example. In the unstimulated state of the cell, eIF4E is held inactive by its binding protein, 4E-BP1. When a stimulus for protein synthesis is received, 4E-BP1 undergoes phosphorylation in a hierarchical manner. Thus, Thr^{37} and Thr^{46} seem to be phosphorylated first (47) followed by Ser^{70} and Ser^{65}. Phosphorylation at the latter site may also be

affected by Ser^{101} phosphorylation (46, 102, 150). Phosphorylation of 4E-BP1 releases eIF4E to bind the mRNA cap and promote translation. The other binding protein of eIF4E, eIF4G, facilitates eIF4E in binding to the cap. The binding sites on eIF4E for 4E-BP1 and eIF4G share the same sequence, and the two proteins compete with each other for binding to eIF4E (92). There are other binding proteins for eIF4E such as maskin (139) and cup, which are tethered to the mRNA through their association with protein linkers. They also exert an inhibitory effect on cap binding activity of eIF4E. Binding interactions among the factors that regulate the initiation phase can also affect efficiency of translation. For example, binding of eIF4G, which interacts with the 5′-end of the mRNA, with PABP, which binds the poly(A) sequence at the 3′-end of the mRNA, serves to promote circularization of the mRNA; this is believed to improve the efficiency of translation (147, 151) (Fig. 1).

Inhibitory RNAs. Human genome codes for many RNAs that do not encode proteins. These noncoding RNA species have important functions in regulation of protein synthesis. There are three important types of endogenous small RNAs that participate in regulation of mRNA: the small interfering RNAs (siRNAs), miRNAs, and piRNAs. The siRNAs are between 20 and 25 nucleotides long and are derived from double-stranded RNAs, which are products of coming together of sense and antisense strands of RNA derived from DNA sequences. The double-stranded RNA precursors exit the nucleus and are digested by Dicer, a cytoplasmic cleaving enzyme belonging to the RNase III family (38, 49). The fragmented double-stranded RNAs form a complex called the RNA-induced silencing complex (RISC) with many proteins that contain double-stranded RNA-binding domains (38); prominent among these proteins is the Ago protein, belonging to the Argonaute family. There are four members in the mammalian Argonaute family (38). The RISC processes double-stranded siRNA to remove passenger strand and leave the antisense strand intact, which then guides the RISC to bind to the mRNA in areas of strict complementarity. The RISC breaks up the siRNA-mRNA complex in the middle, resulting in degradation of the mRNA and inhibition of its translation (26).

miRNAs are 21 nucleotides long. Their precursors, pri-miRNAs, are produced by RNA polymerase II from specific genes or from introns in specific genes in the mammalian genome (5). The pri-miRNAs are double stranded with imperfect base pairing that endows them with hairpin loop structures. They are processed by a complex of Drosha (an RNase III type endonuclease) and DiGeorge Syndrome critical region gene 8 to form ~70-nucleotide long pre-miRNA, which is transported out of the nucleus in combination with exportin 5 protein. Upon arrival in the cytoplasm, miRNA combines with proteins like TAR RNA-binding protein and Ago to form micro ribonucleoprotein (miRNP), also called miRNA-induced silencing complexes. The double-stranded miRNA is processed into single strands of ~21 nucleotides, one of which is degraded and the other is maintained for function; this contains the specific seed sequence of two to eight nucleotides. The mature miRNA binds with roughly complimentary sequences in the 3′-UTR of target mRNA. However, recent reports have identified target sequences in the coding regions of specific genes, including the exon-exon junctions (40, 142). The miRNP promotes fragmentation of mRNA. There are additional mechanisms by which miRNA inhibits protein synthesis. These involve inhibition of mRNA translation. It appears this inhibition applies to translation of capped mRNAs with poly(A) tails (148) and not IRES-containing mRNAs (65, 112). *let-7* and *miR122* miRNAs have been shown to inhibit the initiation phase of translation (65, 112). Although the mechanism by which miRNAs inhibit cap-dependent translation is not currently well understood, suggestions include competition for the mRNA cap between eIF4E and the Ago proteins (80), disruption of the formation of the 80S ribosomal complex (100, 143), inhibition of elongation phase, and, proteolysis of nascent peptide being synthesized during translation (38). It is possible that a specific mechanism of translation inhibition will depend on the individual miRNA. For instance, mechanism of inhibition of mRNA translation by *GW182* miRNA has been reported to be independent of eIF6, which prevents ribosomal assembly; it appears to involve AGO1 protein and *GW182* interaction (31). Recent investigations have shown that a single miRNA can regulate synthesis of many proteins by inhibiting the actions of the corresponding mRNAs (3, 126). Recent findings have extended the scope of miRNA regulation of gene expression to stimulation of translation of mRNAs. During cell cycle arrest, the AU-rich elements in the 3′-UTR of tumor necrosis factor (TNF)-α mRNA recruit Ago and fragile X mental retardation protein1 under the control of *miR369–3*, resulting in promotion of translation (145). Inhibition or stimulation of translation by miRs such as *let-7* appears to depend on whether the cells are undergoing proliferation or arrest.

Parallels have been drawn between the mechanisms of action of transcription factors (TFs) and miRNAs (61). Both are pleiotropic in that each individual TF or miRNA binds many different targets. Both TFs and miRNAs work in a cooperative and combinatorial manner with other TFs and miRNAs; unique combinations of TFs and miRNAs contribute to cell specificity in gene expression, i.e., why some genes are expressed only in specific cell types and repressed in others. The access of TF to the DNA can be regulated by masking their binding sites by nucleosome and that of miRNA by RNA-binding proteins or by secondary structure of the mRNA. Finally, it is important to note that miRNAs can affect the expression of TFs and therefore the transcriptional processes regulated by such TFs (61). This extends the scope of miRNA action to transcription in addition to translation. In contrast to TF, gene regulation by miRNA offers some unique "advantages." Unlike regulation of transcription, which takes some time depending on production and availability of TF through its own gene regulation, the miRNA can affect production of gene product, protein, by immediately binding to the 3′-UTR of mRNA. This action is also reversible by merely disengaging the miRNA from mRNA (61).

The piRNA pathway is of importance in development of germ cells although the precise mechanism of their action is not understood.

Recent investigations have revealed an important role played by miRNA in renal function and disease. Targeted deletion of Dicer in the podocyte resulted in proteinuria and rapid decline in renal function (55, 60, 134). This was associated with increased expression of more than a hundred genes in glomeruli of Dicer knock out mice coinciding with proteinuria. These data suggest that miRNA regulation of gene expression is a determinant of glomerular permselectivity function, and their lack can lead to glomerulosclerosis. Other investigators have

reported that renal parenchyma is rich in expression of *miRs 192, 194, 204, 215*, and *216* (141). Kato and associates (78) have provided a glimpse into the function of *miR-192*. Transforming growth factor-β (TGF-β) increases synthesis of collagen type 1 α_2-chain in mesangial cells by decreasing the expression of an E box repressor known as Smad-interacting protein (Sip1); the decrease in Sip1 expression was shown to be due to an increase in *miR-192*. Corresponding to these in vitro data, the authors found increases in *miR-192* expression in association with TGF-β and collagen type 1 α_2 in the renal parenchyma in rodents with type 1 or type 2 diabetes.

Signaling Regulation of mRNA Translation

As mentioned above, phosphorylation by kinases and dephosphorylation by phosphatases play an important role in regulation of mRNA translation. Detailed reviews of signaling regulation of translation have been published recently (76, 113). We will review signaling regulation of mRNA translation in the context of kidney disease and physiology (Fig. 3).

Diabetic nephropathy

Phosphatidylinositol 3-kinase, protein kinase B pathway. Diverse stimuli for kidney growth such as high glucose, insulin, VEGF, platelet-derived growth factor (PDGF) or insulin-like growth factor-I (IGF-I) promote activation of phosphatidylinositol 3-kinase (PI 3-kinase), a lipid kinase located in the plasma membrane (7, 18–20, 34, 86, 96, 124, 128–130, 146). In the case of receptor tyrosine kinases such as receptor for insulin or IGF-I, autophosphorylation of the receptor promotes binding of a docking protein such as the insulin receptor substrate-1 (IRS-1). Specific tyrosine residues on IRS proteins undergo phosphorylation by the receptor and serve as binding sites for src homology 2 domain containing proteins such as the p85 regulatory subunit of PI 3 kinase (type 1). VEGF promotes IRS-1 binding to VEGF type 2 receptor in renal proximal tubular epithelial cells and is required for induction of PI 3-kinase activity by that growth factor (130). The catalytic p110 subunit of the activated PI 3-kinase (type 1) promotes phosphorylation at the D3 position of the inositol ring of

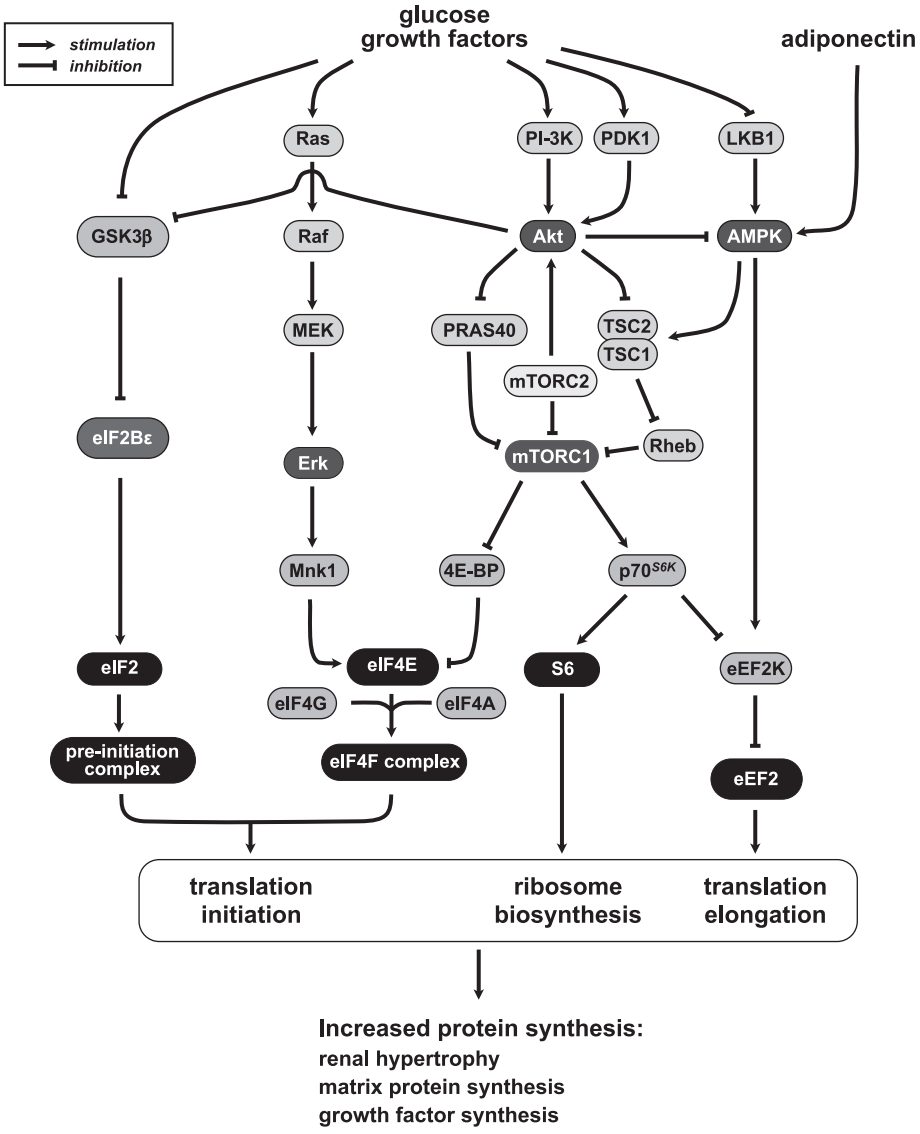

Fig. 3. Signaling regulation of mRNA translation. GSK3β, glycogen synthase kinase 3β; MEK, mitogen/extracellular signal-regulated kinase; Erk, extracellular signal-regulated kinase; PI-3K, phosphatidylinositol 3-kinase; PDK1, phosphatidylinositol-dependent kinase 1; Akt, protein kinase B; PRAS40, proline-rich Akt substrate 40; TSC1 and -2, tuberous sclerosis proteins 1 and 2, respectively; mTORC, mammalian target of rapamycin complex; 4E-BP, 4E-binding protein; AMPK, AMP-activated protein kinase.

plasma membrane-bound inositides such as phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate, resulting in formation of phosphatidyl 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate (PIP3), respectively (19, 21, 94). The renal cortical PI 3-kinase activity is increased in diabetic nephropathy coinciding with renal hypertrophy and matrix accumulation (34). High glucose-induced protein synthesis and matrix protein, laminin synthesis in the GEC, mesangial, and proximal tubular epithelial cells require PI 3-kinase activation (86, 93, 96). The PI 3-kinase product PIP3 is a substrate for a phosphatase, phosphoinositide-3-phosphatase and tensin homolog (PTEN), which is a natural inhibitor of activity of PI 3-kinase (120). Activity of PTEN is reduced in renal cortex at the time of renal hypertrophy in streptozotocin-induced type 1 diabetes; mesangial cells incubated with high glucose also show reduction in PTEN, which appears to be TGF-β dependent (93). PIP3 has high affinity for proteins with pleckstrin homology (PH) domains and recruits them to the plasma membrane. Prominent among PH domain-containing molecules are protein kinase B (Akt), a serine threonine kinase, and phosphatidylinositol-dependent kinase 1 (PDK-1) (41). Akt is held inactive in the cytoplasm by calcium calmodulin (28), inositol 1,3,4,5-tetrakisphosphate (73), and casein kinase-2-interacting protein 1 (144). Following recruitment to the plasma membrane, PDK-1 catalyzes phosphorylation of Akt on Thr^{308} located in the kinase domain (8). Full activation of Akt activation requires additional phosphorylation on Ser^{473}, which is under the control of mammalian target of rapamycin (mTOR) complex (C) 2 (122). Once localized to the plasma membrane, Akt has additional regulators of its activity. Thus adaptor protein-containing PH domain, PTB domain, and leucine zipper motif (101), Akt phosphorylation enhancer (2), and BTPD10 (106) promote its activity, whereas tribbles homolog 3 inhibits it (29). Regulation of Akt occurs in cell type-specific and context-specific manner. Akt activation occurs rapidly in glomerular epithelial cells and proximal tubular epithelial cells incubated with high glucose or high insulin (86, 93, 96); Akt activation has also been reported in the renal cortex of rodents with type 1 or type 2 diabetes at the time of renal hypertrophy, proteinuria, and matrix accumulation (34, 86). Akt activation is required for IGF-I-induced protein synthesis in proximal tubular epithelial cells (127). Akt activates several other pathways in altering protein synthesis; the pathway involving mTOR is particularly important in translation (Fig. 3).

Akt recruits direct and indirect pathways to stimulate activity of mTOR. Direct regulation involves proline-rich Akt substrate 40 (PRAS40) (83). In the basal state, PRAS40 is associated with raptor in mTOR complex 1 (mTORC1) and exerts an inhibitory effect (121). In insulin-treated cells, phosphorylation of PRAS-40 by Akt relieves this inhibition and promotes mTOR activation (121, 147). Preliminary observations show that, in mesangial cells, high glucose-induced PRAS40 phosphorylation is Akt-dependent and is required for the increase in protein synthesis (Dey, Kasinath, Ghosh Choudhury, unpublished data). The indirect pathway involves hamartin and tuberin also known as tuberous sclerosis proteins 1 and 2 (TSC-1, TSC-2), respectively. TSC-2 and TSC-1 form a heterodimer (48); TSC2 serves as a GTPase-activating protein that inhibits the actions of Ras homolog enriched in brain (Rheb) (71). Rheb promotes mTOR activity when associated with GTP (13). Activation of Akt results in phosphorylation of TSC-2, inhibition of its dimerization with TSC-1, and activation of Rheb and thus of mTOR (70). The precise mechanism underlying Rheb association with mTOR complex 1 (mTORC1) is being elucidated. In the setting of stimulation by amino acids, GTP-bound Rag GTPases promote localization of Rheb and mTOR to the same compartment in the cell (79, 121). This still does not answer the precise mechanism of stimulation of mTORC1 activity by Rheb although it sheds light on their association (156). A recent in vitro study has shown that Rheb promotes binding of 4E-BP1 to mTORC1 (125). Another membrane-bound protein, FKBP38, appears to bind mTORC1 and inhibit its activity; it dissociates from mTORC1 when amino acids stimulate Rheb binding to mTORC1 (4); however, recent data have questioned the requirement of FKBP38 in interactions between Rheb and mTORC1 (125). It is important to note that AMP-activated protein kinase (AMPK) is also upstream of TSC2 and modulates mTOR activity. This will be discussed later.

mTOR. Both initiation and elongation phases of translation are under the control of mTOR. A large protein belonging to the PIKK family, mTOR exists in two complexes that have diverse functions. By combining with raptor and GbetaL, it forms mTOR complex1 (mTORC1), which has two direct substrates, p70S6 kinase and 4E-BP1. These proteins contain sequences, e.g., FEMDI (Phe-Glu-Met-Asp-Ile), called TOR signaling motifs (TOS) which facilitate mTORC1 binding (52, 107). Activation of p70S6 kinase by mTOR occurs by phosphorylation on Thr^{389}; activated p70S6 kinase catalyzes phosphorylation of ribosomal proteins such as S6, an important event in the initiation phase of translation (72). The mTORC1 also phosphorylates 4E-BP1 on Ser^{65}, leading to its dissociation from eIF4E, a critical step in the initiation phase; phosphorylation of $Thr^{37/46}$ on 4E-BP1 may also be under control of mTORC1 except that the RAIP (Arg-Ala-Ile-Pro) motif is involved rather than the TOS motif (113, 149). Increase in 4E-BP1 phosphorylation is noted in proximal tubular and mesangial cells incubated with insulin, high glucose, IGF-I, ANG II, and VEGF in association with augmented protein synthesis and matrix protein increment; correspondingly, renal cortical homogenates from diabetic rodents also show an increase in 4E-BP1 phosphorylation at the time of renal hypertrophy and matrix accumulation (7, 24, 34, 93, 96, 127–129). mTORC2 is formed by binding of mTOR with rictor, Sin1, and GbetaL; an additional binding protein, proline rich protein 5 has also been identified as a component of mTORC2 (153). The functions of mTORC2 are not well understood but may include regulation of actin. As mentioned above, it is involved in Ser^{473} phosphorylation of Akt (122). Recent investigations have suggested that mTORC2 is involved in activation and stability of Akt and PKC (32, 66). Very interestingly, recent studies in mesangial cells have indicated that mTORC2 may exert a tonic inhibition on mTORC1 (24).

The Elongation phase of translation is also regulated by mTOR. Phosphorylation of eEF2 on Thr^{56} is under the control of eEF2 kinase, a calcium/calmodulin-dependent kinase. Stimulation of p70S6 kinase by mTORC1 results in inhibition of eEF2 kinase activity by phosphorylation of Ser^{366} (116). This results in reduction in the phosphorylation of eEF2 on Thr^{56}, which facilitates the elongation phase (116). High glucose and high insulin induce dephosphorylation of eEF2 and augment

Ser^{366} phosphorylation in proximal tubular epithelial cells and glomerular epithelial cells (86, 124). Such changes are also seen in the renal cortex of mice with type 2 diabetes coinciding with renal hypertrophy and laminin accumulation (124). Administration of rapamycin inhibits elongation phase reactions and limits both renal hypertrophy and laminin accumulation in mice with type 2 diabetes without affecting the elevated glucose levels (124). Other investigators have reported on the ameliorative effects of rapamycin on the course of renal disease in type 1 and type 2 diabetes (91, 119). The role of phosphatases in regulation of eEF2 phosphorylation is not extensively studied.

In addition to the canonical PI 3-kinase-Akt axis, other signaling pathways also participate in regulation of mTOR affecting protein synthesis in the context of diabetes.

AMPK. AMPK is a heterotrimeric protein made of $\alpha_{1/2}$-, $\beta_{1/2}$-, and $\gamma_{1/2/3}$-subunits; one isoform of each subunit is required for its activity (12, 53). Its activity depends on phosphorylation of Thr^{172} on the α-subunit (57). AMPK inhibits energy-consuming reactions such as synthesis of fatty acids and sterols and activates ATP-generating processes such as fatty acid oxidation and glycolysis (103). An increase in AMP content and decrease in ATP content stimulate AMPK activity (12). Although its control of carbohydrate and lipid metabolism has been well studied, the role of AMPK in protein metabolism and in renal physiology is still unclear. Lee et al. (86) have reported that diabetes-induced renal hypertrophy in rodents with type 1 or type 2 diabetes is associated with reduction in AMPK phosphorylation and activity. A similar reduction was also seen in glomerular epithelial cells in association with high glucose-induced increase in protein synthesis and hypertrophy. In glomerular epithelial cells exposed to high glucose, reduction in AMPK was associated with an increase in mTORC1 activity, increase in phosphorylation of p70S6 kinase and 4E-BP1, and reduction in eEF2 phosphorylation (86). These changes were seen in association with high glucose-induced augmented protein synthesis in the GEC. Stimulation of AMPK by metformin or 5-aminoimidaxole-4-carboxamide-1-β-D-ribofuranoside (AICAR) resulted in inhibition of high glucose-induced stimulation of initiation and elongation phase reactions and of protein synthesis. Administration of metformin and AICAR to rats with streptozotocin-induced diabetes resulted in amelioration of renal hypertrophy without changes in plasma glucose (86). Additionally, the PI 3-kinase-Akt axis has been shown to reduce AMPK phosphorylation (15, 82). Investigation of signaling pathways has placed AMPK downstream of Akt but upstream of mTOR (86). The mechanism by which AMPK inhibits mTORC1 involves TSC2. AMPK phosphorylates TSC2 on $Ser^{1227,1345}$ (71) and augments its activity to inhibit Rheb as described above, culminating in inhibition of mTORC1 and protein synthesis.

The signals in the diabetic kidney that alter AMPK activity are not known; changes in AMP and ATP could not account for reduction in AMPK activity induced by high glucose in the GEC (86). Other potential regulatory factors include LKB-1, high osmolality (43, 44), and calcium/calmodulin-dependent kinase kinase (9). LKB-1 is a kinase that phosphorylates Thr^{172} on the α-catalytic subunit of AMPK (154). Preliminary observations have shown that high glucose reduces LKB-1 activity in the glomerular epithelial cells by promoting its acetylation (Lee and Kasinath, unpublished data). Recently, adiponectin, an adipokine, has been found to regulate AMPK activity in the kidney. Adiponectin null mice have proteinuria, and exogenous administration of adiponectin corrects this abnormality by stimulating AMPK activity (131). These fascinating data show that AMPK has several functions in the kidney. In addition to being a constitutive inhibitor of protein synthesis in the kidney, it also serves as a mediator of adiponectin regulation of permselectivity function of the glomerulus. AMPK has another potential role in regulating translation: it can phosphorylate eEF2 kinase on Ser^{398} (11) and activate it. This would result in inhibition of the elongation phase of translation. Thus AMPK can regulate both the initiation and elongation phases of translation. Data from studies of Lee et al. (86) and Sharma et al. (131) show that AMPK could be a potential target for intervention in diabetic nephropathy.

One of the puzzling issues in the field of signal transduction is how activation of a signaling kinase can result in distinct responses in different tissues. For instance, AMPK is involved in regulation of lipid and protein metabolism. It appears to recruit distinct mediators for its regulation of lipid vs. protein metabolism. AMPK phosphorylates acetyl-CoA carboxylase (ACC), which mediates AMPK inhibition of fatty acid synthesis in the liver. On the other hand, phosphorylation of tuberin (TSC2 gene product) by AMPK leads to inhibition of protein synthesis. One may conceive of a nodal pattern where AMPK-catalyzed phosphorylation of ACC leads to changes in lipid metabolism, whereas that of TSC2 leads to inhibition of protein synthesis. This still does not clarify the issue of what determines specificity of tissue responses to AMPK, i.e., how does AMPK selectively regulate lipid metabolism in the liver vs. protein synthesis in the kidney? There might be additional regulatory factors that dictate tissue-specific responses, and these need to be identified in the future.

Glycogen synthase kinase 3β. Glycogen synthase kinase 3β (GSK3β) is well known for its regulation of carbohydrate metabolism (30). Although it is known to phosphorylate the initiation factor eIF2Bε and inhibit its activity, its potential role in regulation of protein metabolism in the kidney has not been studied. Recently, Mariappan et al. (98) showed that GSK3β activity is inhibited by induction of phosphorylation on Ser^{9} by high glucose and high insulin in proximal tubular epithelial cells in association with stimulation of the matrix protein, laminin-β_1 and fibronectin; high glucose and high insulin were employed as pathogenic factors for kidney disease in type 2 diabetes. This was associated with reduction in phosphorylation of both eIF2Bε and eEF2. Increase in phosphorylation of GSK3β, which would inhibit its activity, was found in renal cortical homogenates of mice with type 2 diabetes coinciding with laminin accumulation (98). One of the critical reactions in the initiation phase of translation is the binding of eIF2-GTP to the activated methionyl-tRNA and formation of the preinitiation complex with 40S ribosomal subunit (113). Reduction in eIF2Bε phosphorylation promotes its activity as a guanine nucleotide exchange factor, facilitating loading of GTP to eIF2 (152). Thus, by inhibition of GSK3β, high glucose and high insulin promote preinitiation complex assembly during the initiation phase of translation. Thus GSK3β is a natural inhibitor of protein synthesis in renal cells; diabetes induces a reduction in its activity in association with renal hypertrophy and matrix protein accumulation. Whether GSK3β serves as a

viable target for intervention in diabetic nephropathy remains to be determined.

Mitogen-activated protein kinases. Of the various kinds of mitogen-activated protein (MAP) kinases, extracellular signal-regulated kinase (Erk) 1/2 type MAP kinase is closely associated with growth and protein synthesis. Both high glucose and high insulin, factors of importance in type 2 diabetes, induce Erk activation in renal cells and tissues in vivo and in vitro models of diabetic nephropathy (7, 34, 97, 128, 130). Renal injury by high glucose is mediated in part by growth factors such as ANG II, TGF-β, and VEGF. VEGF expression is increased in renal cortex of mice with type 1 or type 2 diabetes (128), suggesting a role for VEGF in renal hypertrophy. In support of this notion, VEGF stimulated protein synthesis and induced hypertrophy in proximal tubular epithelial cells in culture (ibid). This effect involved recruitment of VEGF type 2 receptor, IRS-1, PI 3-kinase, and the Akt pathway culminating in 4E-BP1 phosphorylation (127, 128). VEGF also promoted Erk phosphorylation, which was mediated by the Ras-Raf-mitogen/extracellular signal-regulated kinase pathway (97). Activation of Erk led to induction of activity of MAP kinase interacting kinase 1 (Mnk-1), which directly controls eIF4E phosphorylation. Thus VEGF-induced eIF4E phosphorylation on Ser^{209} was Erk dependent and contributed to the increase in protein synthesis (97). VEGF promoted 4E-BP1 phosphorylation, which led to dissociation from eIF4E and association of the latter with eIF4G (97), an important reaction in the initiation phase of translation. Similarly, TGF-β-induced protein synthesis and hypertrophy in mesangial cells also requires Ser^{209} phosphorylation on eIF4E (Das, Kasinath, Ghosh Choudhury, unpublished data). Erk has also been implicated in phosphorylation of 4E-BP1 (7). Another MAP kinase, p38 kinase, has been implicated in mRNA stability (25).

Role of translation in synthesis of growth factors and their actions. Recent investigations have revealed important roles played by mRNA translation in both elaboration of growth factors and their actions. Content of TGF-β mRNA is augmented by high glucose in proximal tubular epithelial cells; translation of the mRNA into TGF-β protein requires a cooperative action of PDGF (42). Increase in gene expression of a growth factor, growth arrest-specific gene6 (GAS6) and Axl, a receptor for GAS6, is associated with diabetes-induced renal hypertrophy (104). High glucose-induced 4E-BP1 phosphorylation via activation of PI 3 kinase-Akt-mTOR appears to depend partly on activation of GAS6-Axl expression (104). ANG II has assumed a central role in pathogenesis of diabetes-related kidney injury (117). ANG II promotes expression of several growth factors, including TGF-β and VEGF. VEGF expression is increased in the kidney in mice with type 1 or type 2 diabetes (128), and its neutralization ameliorates several renal abnormalities in diabetes (27, 39). Feliers and associates (33) have reported that ANG II promotes VEGF expression within minutes of incubation in proximal tubular epithelial cells by promoting its translation. Phosphorylation of 4E-BP1 via the PI 3 kinase-Akt-mTOR axis is required for these changes and appears to depend on generation of reactive oxygen species generated by the NAD(P)H oxidase system rather than the mitochondrial system (36). ANG II regulation of translation of VEGF also involves events occurring in the 3′-UTR. ANG II promotes the binding of heterogeneous nuclear ribonucleoprotein K (hnRNPK) to the 3′-UTR of VEGF mRNA, which appears to augment translation (37). The ability of hnRNPK to promote VEGF mRNA translation requires phosphorylation by PKC delta, which is itself under the control of src tyrosine kinase (123).

Role of translation in renal matrix metabolism in diabetes. Accumulation of matrix proteins in glomerular and tubulointerstitial compartments is associated with loss of renal function in diabetes (99). Because type 2 diabetes is associated with hyperinsulinemia in addition to hyperglycemia, effects of high glucose and high insulin on metabolism of laminin-β_1, a matrix protein, has been investigated. Ha et al. (51) reported that laminin accumulation in renal cortex of mice with type 2 diabetes was not accounted for by increment in the mRNA content of the respective chains, suggesting that an increase in efficiency of translation could be a mechanism contributing to accumulation of laminin. Mariappan et al. (96) have provided in vitro evidence for the role of translation. They observed that high glucose or high insulin promoted laminin-β_1 synthesis in renal proximal tubular epithelial cells within minutes without changes in laminin-β_1 mRNA content (96). The rapid increment in high-glucose- or high-insulin-treated cells was associated with phosphorylation of 4E-BP1 and eIF4E, reduction in their association, and increment in association between eIF4E and eIF4G (96). High glucose and high insulin recruited the canonical PI 3-kinase-Akt-mTOR pathway to induce 4E-BP1 phosphorylation on Ser^{37} and Ser^{46}, which was needed for rapid phase increment in laminin-β_1 (96). Because rapamycin, an mTOR inhibitor, was able to inhibit laminin-β_1 accumulation induced by high glucose and high insulin, its effect on laminin expression was examined in mice with type 2 diabetes. At 3 wk of hyperglycemia and hyperinsulinemia in *db/db* mice, an increase in laminin-β_1 protein was evident without any increase in its mRNA; the elongation phase of translation was stimulated in the renal cortex as shown by the decrease in Thr^{56} phosphorylation of eEF2, increase in Ser^{366} phosphorylation of eEF2 kinase, and increase in Thr^{389} phosphorylation of p70S6 kinase, the latter being an index of mTOR activation (124). Administration of rapamycin reduced the laminin-β_1 increment and renal hypertrophy in diabetic mice and was associated with inhibition of elongation phase reactions; rapamycin did not affect blood glucose levels in diabetic mice. These data show that translation is an independent mechanism of accumulation of laminin in diabetic kidney and that kinases such as mTOR involved in regulation of translation can be therapeutic targets in diabetic nephropathy.

It is worth noting that renal injury in the context of diabetes involves stimulation of both general protein synthesis where structural proteins are upregulated and induction of selective translation of specific transcripts such as matrix protein laminin in type 2 diabetes. Whereas the renal hypertrophy due to the increase in general protein synthesis occurs quite early in diabetes, translation-driven laminin synthesis seems to occur later, coinciding with an increase in synthesis of other matrix proteins such as type IV collagen and fibronectin. It is also possible that an increase in matrix proteins also contributes to hypertrophy of the kidney, e.g., mesangial matrix expansion contributing to glomerular hypertrophy.

Compensatory renal hypertrophy. Loss of one kidney leads to enlargement of the remaining kidney largely by the process of hypertrophy, i.e., increase in protein and RNA content (64). The mechanism of renal hypertrophy includes events in cell

cycle regulation (35, 89). Because augmented protein synthesis contributes to renal hypertrophy, it is logical to anticipate stimulation of translation. Chen et al. (17) have explored translation events occurring in the kidney undergoing compensatory growth. Rapid onset of phosphorylation of ribosomal protein S6 and 4E-BP1 could be seen following removal of the opposite kidney; these indexes suggested activation of mTOR because p70S6 kinase that phosphorylates protein S6 and 4E-BP1 are direct substrates of mTORC1. Rapamycin, a selective mTOR inhibitor, abolished mTOR activation and inhibited the extent of growth of the kidney. Thus mTOR activation is central to the physiological adaptation of the kidney to loss of renal mass. Uninephrectomy also promotes interleukin (IL)-10 and TGF-β expression in the adapting kidney; administration of AS101, an immunomodulator that inhibits IL-10 expression, partly inhibits compensatory renal growth (137). In compensatory growth of tubules following uninephrectomy, elevated levels of TGF-β correlated better with the increase in amount of cell cycle protein p57kip2 bound to cyclin E complex rather than with p21waf1 or p27kip1 (138). Regulation of translation pathways by IL-10 and TGF-β in compensatory growth has not been explored. Compensatory mechanisms employed by the surviving kidney depend upon whether the loss of the contralateral kidney is due to pathology such as obstruction or due to loss from uninephrectomy of a healthy kidney (56). In both conditions, growth inhibitory genes are suppressed. However, there are differences between the conditions as well. Whereas in the case of unilateral obstruction, transport genes are upregulated, following uninephrectomy cell cycle and metabolism genes were activated. More work is needed to tie these genes to the process of augmented translation involved in compensatory renal growth.

Although distinct from compensatory growth, fetal growth is another physiological context in which kidney volume increases. Recently, Geelhoed et al. (45) studied determinants of kidney growth from fetal life until two years of age. They found that anthropometric features such as maternal body weight and height; fetal head and abdominal dimensions; and fetal weight and length at third trimester correlated positively with kidney size. In addition, in the fetus, distribution of blood between brain and kidney also affected kidney volume. Preferential blood flow to the brain in the fetus was associated with smaller kidney size. Weight gain after birth also positively affected the infant's kidney growth. Nutritional status of the mother may be associated with sufficiency of amino acids and induce mTOR activity in the fetal kidney and promote optimal translation; similar correlation may hold between optimal nutrition-associated weight gain in the postnatal period and kidney growth. Smaller kidneys and fewer nephrons in the developing fetus and early childhood could have implications for "nephron dosing" and susceptibility to kidney disease in later life (10).

Translation in endoplasmic reticulum stress in the kidney. Following peptide synthesis by the ribosome, maturation, and folding of the protein occur in the endoplasmic reticulum (ER). In states of stress e.g., exposure to inhibitors of protein synthesis, metabolic syndrome, misfolded proteins are retained in the ER. ER stress is defined as the consequence of a mismatch between the load of unfolded and misfolded proteins in the ER and the capacity of the cellular machinery that copes with that load (118). ER stress leads to activation of expression of genes for ER chaperones; the signaling pathway mediating this response is called the unfolded protein response (UPR) (118). The ER contains transmembrane proteins with domains facing the ER lumen that act as sensors of unfolded proteins. Activation of UPR via these proteins is not completely understood but may involve direct activation of the sensors by unfolded proteins or dissociation of the sensors from a ER chaperone protein called glucose response protein 78 (GRP78), also called immunoglobulin binding protein. Response to ER stress entails inhibition of general synthesis of proteins; however, selected proteins are still synthesized. This may represent an important adaptation by the cell, allowing for conservation of energy when faced with stress; however, prolonged ER stress and inability of the cells to adequately respond to it can result in apoptosis, thus removing a source of toxic unfolded proteins. In ER stress, inhibition of global protein synthesis is achieved by phosphorylation of eIF2α by RNA-activated protein kinase like ER kinase (PERK). Following detection of unfolded proteins, PERK undergoes dimerization and autophosphorylation. Activated PERK catalyzes Ser^{51} phosphorylation of its substrate eIF2α (95). This inhibits eIF2B, a guanine nucleotide exchange factor, which normally facilitates loading of GTP on eIF2, a part of the preinitiation complex. Thus phosphorylation of eIF2α inhibits activities of eIF2B and eIF2, leading to inhibition of general protein synthesis (54). Phosphorylation of eIF2α via PERK activation also results in an increase in expression of activating transcription factor (ATF) 4, a TF that promotes expression of genes encoding amino acid transporters, X-box protein 1 (XBP-1), and antioxidant response genes (118). Phosphorylated eIF2α is also reported to activate nuclear factor-κB (74).

In addition to PERK, two other pathways, inositol-requiring enzyme 1 (IRE1) and ATF6, are also activated. IRE1 undergoes autophosphorylation and its ribonuclease function that is dormant in unstressed cells is now activated (135). Ribonuclease activity of IRE1 facilitates removal of an intron from the precursor mRNA of XBP-1. The spliced mRNA is translated into XBP-1, which functions as a TF resulting in an increase in expression of genes involved in ER-associated degradation of unfolded proteins (155). In *Drosophila*, IRE1 activation may also lead to degradation of preformed mRNA, contributing to inhibition of protein synthesis (62). ATF6 is the other transmembrane sensor of unfolded proteins in the ER. Its activation by dissociation from GRP78 results in its movement to Golgi where it is sequentially cleaved by site-1 and -2 proteases (Sip-1, Sip-2); the resulting peptide fragment translocates to nucleus and is thought to induce expression of genes involved in UPR, similar to the IRE1 pathway (1, 58, 133).

In the kidney, ER stress has been reported in Heymann nephritis, a model of membranous nephritis (23), cisplatinum-associated acute tubular necrosis (90), puromycin aminonucleoside nephropathy (105, 108), and following overexpression of megsin, a serine protease inhibitor (69). In the rat model of mesangial proliferative glomerulonephritis induced by anti Thy-1 antibody, glomerular PERK phosphorylation and phosphorylation of its substrate eIF2α are increased, suggesting inhibition of mRNA translation; pretreatment with inducers of ER stress such as tunicamycin or thapsigargin appears to attenuate this response (68). More recently, markers of ER stress such as GRP78, HYOU1 (ORP150), and XBP-1 have been reported as being increased from kidney biopsies of

humans with established diabetic nephropathy but not mild diabetic nephropathy. Because similar changes were seen in renal tissue in patients from minimal change nephritic syndrome, albuminuria could induce ER stress in renal epithelial cells; this was confirmed in tubular epithelial cells in vitro (88). ER stress in kidney diseases has been elegantly reviewed recently (67).

Translation in acute kidney injury. TNF-α is an important mediator of acute kidney injury induced by cisplatin. Recent work has shown that an increase in TNF-α expression in the cisplatin-injured kidney may be dependent on the agent promoting the translation of the cytokine by stimulating eIF4E phosphorylation. By employing selective inhibitors, the investigators suggested that p38 MAP kinase and Erk activation by cisplatin led to activation of Mnk-1, the kinase that phosphorylates eIF4E on Ser^{209} (114).

Conclusion

Ambient levels of mRNA do not often correlate with the levels of the proteins they code for, suggesting the two entities should be studied separately. Regulation at the level of translation is suggested when *1*) the protein level increases in the absence of rise in the level of its mRNA and *2*) when there is rapid increment in synthesis of the protein within minutes of application of stimulus. It is possible that many instances of rapid regulation of a protein by translation are simply missed because the early time course was not studied. Translation is strictly controlled by the coordinated regulation of several regulatory factors affecting initiation, elongation, and termination stages and by binding proteins, small inhibitory RNAs, and signaling reactions. These pathways of regulation can be harnessed to generate agents that stimulate or inhibit protein synthesis. One advantage of recruiting translation is that regulation can be effected and terminated rapidly in contrast to transcription. Work reviewed here supports the view that signaling molecules involved in translation can potentially serve as therapeutic targets, e.g., AMPK, mTOR, and GSK3β. Additionally, studies on translation can provide insight into kidney development, its physiological function, and response of the kidney to injury in a wide variety of disorders.

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