Signal Transduction Pathways That Regulate Eukaryotic Protein Synthesis*

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The last several years have witnessed an explosion in the published literature on two topics, the pathways that transduce extracellular signals to their intracellular targets and modification of the core translational apparatus in response to these signals. Most of these pathways result in cell growth and cell division. Synthesis of the entire complement of proteins is necessary to double the cell size, but synthesis of the so-called “growth-regulated” proteins (1) is needed for cell division. This article summarizes recent advances in our understanding of how a single mitogenic stimulus can simultaneously lead to an increase in both global and growth-regulated protein synthesis.

**Mechanism of Protein Synthesis**

The three stages of protein synthesis are catalyzed by initiation, elongation, and release factors (Ref. 2; a guide to current and previous nomenclature can be found in Ref. 3). A ternary complex of eIF2-GTP-Met-tRNA, 1 binds to the 40 S ribosomal subunit to form the 43 S initiation complex (Fig. 1). The eIF4 factors plus poly(A)-binding protein recognize the 5′-terminal cap or 3′-terminal poly(A) tract of mRNA, unwind mRNA secondary structure, and transfer it to the 43 S initiation complex, resulting in the 48 S initiation complex. Scanning for the first initiation codon in good sequence context requires eIF4A and the presence of eIF1 and eIF1A (4). Then eIF5 stimulates GTP hydrolysis by eIF2, after which the initiation factors are replaced by the 60 S subunit to form the 80 S initiation complex. The released eIF2-GDP is recycled to eIF2-GTP by the GEF eIF2B. The first elongator aminocyl-tRNA is brought to the A-site by eIF2B, followed by a cycle of GTP hydrolysis and exchange analogous to that of eIF2. Translocation is catalyzed by eEF2, again with a GTP hydrolysis cycle.

**Signaling Intermediates Involved in Protein Synthesis**

**RTKs**—The binding of growth factors to the extracellular domain of RTKs causes a conformational change that induces oligomerization and activation of the intracellular protein Tyr kinase domain (Ref. 5; Fig. 2). Substrates for the kinase can be either the RTK itself or a separate RKS. The SH2 domains of several different signaling molecules dock to the resulting Tyr(P)s in a sequence-specific manner, thereby activating separate downstream signaling cascades.

**GPCRs**—These receptors are coupled to heterotrimeric G-proteins (6). Dissociation of the G-protein subunits activates AC, PLC, and other downstream effectors. PLC hydrolyzes PtdIns(4,5)P$_2$ to DAG and Ins(1,4,5)P$_3$.

**RKS**—These are docking proteins for downstream effectors of RTKs (7). The best studied RKS are the insulin receptor substrates, which include IRS-1, IRS-2, IRS-3, Gab-1, and p62DOK. Members of the IRS family bind to insulin receptor via an NHL-terminal PH domain and a Tyr(P)-binding domain. The COOH-terminal portions of the proteins contain numerous Tyr phosphorylation sites. IRS-1 alone provides docking sites for PI3-K, SH-PTP2, Grb-2, Fyn, Nck, and Crk.

**SH-PTP2**—This phosphatase contains two SH2 domains, and enzyme activity is maximally activated when both are occupied by Tyr(P)-containing peptides (8). SH-PTP2 is activated by docking to EGF receptor, platelet-derived growth factor receptor, c-kit, insulin receptor, JAK-1, JAK-2, and IRS-3 and may serve to attenuate the Tyr(P) signal in these molecules (7).

**Ras**—This G-protein is bound to the plasma membrane by COOH-terminal prenylation and myristoylation (9). GEF activity is provided by SOS, which associates constitutively with the SH2- and SH3-containing protein Grb-2. The Grb-2-SOS complex is recruited to the plasma membrane by binding to specific Tyr(P)s in IRS-1, IRS-2, Shc, or SH-PTP2 (7). Another GEF, Ras-GEF, is stimulated by Ca$^{2+}$/calmodulin (CaM) downstream of GPCR (10). The hydrolysis of GTP by Ras is stimulated by GTPase-activating proteins such as p120$^{C^T}$ and NF1 (9).

**MAPKs**—Ras-GTP activates the Ser/Thr kinase Raf-1 by recruiting it to the plasma membrane. Raf-1, in turn, phosphorylates and activates MEK1 and MEK2. The MEKs are dual specificity kinases, phosphorylating both Thr and Tyr residues in ERK1 and ERK2 (p42 and p44 MAPKs).

**PI3-K**—This kinase is composed of a catalytic subunit and a...

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1 The abbreviations used are: eIF, eukaryotic initiation factor; aa, amino acids; AC, adenylate cyclase; CaM, calmodulin; DAG, diacylglycerol; eEF, eukaryotic elongation factor; eEF2K, eEF2 kinase; eIF4F, eIF4E, eIF4G, eIF3, eIF1, eIF5, eIF2B, eIF2A, eIF2, eIF1A, eIF1, eIF2, eIF2B, A, eIF5, eIF4E, eIF4F, G, eIF4G, eIF1, eIF5, eIF2B, S6, eIF2B, S6, ribosomal protein S6. Those factors shown in color are targets of the signaling pathways in Fig. 2.

**FIG. 1.** Eukaryotic protein synthesis and sites of action for initiation and elongation factors. Factors are abbreviated as 2, eIF2, 2B, eIF2B, A, eIF4A, E, eIF4E, 4F, eIF4F, G, eIF4G, E1, eEF1, E2, eEF2, S6, ribosomal protein S6. Those factors shown in color are targets of the signaling pathways in Fig. 2.
SH2-containing regulatory subunit that binds to TyrP's in RTKs and RKS (7). PI3-K is also activated synergistically by direct binding to Ras-GTP (11). PI3-K is a dual specificity kinase that phosphorylates PtdIns at the 3-position and proteins on Ser/Thr residues (12). The lipid phosphorylation signal activates PDK and PKB, and the protein phosphorylation signal activates MAPK (13). Both activities are inhibited by wortmannin and LY294002 (14).

**PKC**—These recently discovered kinases, with at least four isoforms, bind to and are activated by PtdIns(3,4,5)P3 by their COOH-terminal PH domains (15, 16).

**PKB** (Akt, RAC-PK)—PKB exists in at least four isoforms (α, β1, β2, γ) and is activated by both RTKs and GPCR. In the former case, PI3-K is involved (17), but in the latter, there are both PI3-K-dependent (18) and -independent (19) pathways. PKB is targeted to the plasma membrane by direct binding to PtdIns(3,4)P2 and PtdIns(3,4,5)P3 through its PH domain (20), where it is activated by phosphorylation at Thr-308 by PDK (15).

**PKC**—There are at least 10 isoforms of PKC (α–ξ) that differ in responsiveness to phospholipids and Ca2+ (21). Classical PKCs (α, β, and γ) are activated and eventually down-regulated by phorbol esters, which are structural analogs of the physiological signal DAG, but atypical isoforms (λ and η) are not. Insulin activates both classical and atypical isoforms (22, 23). PKCζ is activated downstream of PI3-K (24) via direct phosphorylation at Thr-410 by PDK1 (25).

**Ca2+**—Changes in cytosolic Ca2+ levels can occur by at least two mechanisms (26). First, GPCR operate Ca2+ influx from the extracellular space. Second, Ins(1,4,5)P3, released in PtdIns(3,4,5)P3 through its PH domain (20), where it is activated COOH-terminal PH domains (15, 16).

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the interaction between 40 S subunits and mRNA (62).

**eEF1—**The first of the elongation factors is composed of α, β, γ, and δ subunits. Various subunits are phosphorylated in vitro by several kinases, including casein kinase II, multipotential S6 kinase, and PKC (36). In the latter two cases, these phosphorylations result in a stimulation of elongation and GDP/GTP exchange, respectively.

**eEF2—**This elongation factor is inhibited by phosphorylation via eEF2 kinase on Thr-56 and Thr-58, the phosphorylation of the latter site requiring prior phosphorylation of the former (26, 63).

**Examples of Specific Signaling Pathways Affecting Protein Synthesis**

**Insulin—**Insulin, by far the best characterized effector of protein synthesis, stimulates both initiation and elongation. eEF2α phosphorylation does not change, but eIF2β activity nonetheless increases by the pathway IRS-1 (Tyr-608, -628, and -658; Ref. 64) → PI-3-K (64) → PDK → PKB (65) → GSK-3 (26) → eIF2β (53) (see Fig. 2). However, insulin may activate eIF2β through additional routes, because constitutively active PECCh stimulates general protein synthesis in insulin-dependent manner without activating p70S6k, suggesting that PKB is not involved (23). Binding of SHPTP2 to IRS-1 (Tyr-1172 and -1222) attenuates insulin-stimulated protein synthesis (60). PHAS-I is phosphorylated in response to insulin (58, 57) through a rapamycin-sensitive but MAPK-insensitive pathway (64, 67, 68) that includes PKB (69), indicating that the pathway is IRS-1 (Tyr-608, -628, and -658) → PI-3-K → PDK → PKB → mTOR → PHAS. Phosphorylation of eIF4E by the pathway IRS-1 (Tyr-895) → Grb-2-SOS → Ras → Raf → MEK → ERK → Mnk. (There are numerous other RKS that contribute to insulin stimulation of MAPK, especially Shc.) eIF2α association with eEF2 is enhanced by insulin (70) as is eIF4G phosphorylation by MAPK, especially Shc.) eIF4G association with eIF4E is stimulated by insulin (70) as is eIF4G phosphorylation by MAPK, especially Shc.) eIF4E association with 3

**PKC—**Phosphorylation of PKC (36). In the latter two cases, these phosphorylations result in a stimulation of the activity of eIF2B is the pathway that are involved in cell growth and cell cycle progression (89). These mRNAs are poorly translated in quiescent cells but preferentially stimulated translation of growth-regulated mRNAs with high 5'-UTR secondary structure.

**5'-TOP mRNAs are also differentially regulated in response to extracellular signals (97).** These encode many translational components, including ribosomal proteins, eEF1α, eEF2, and polyA-binding protein. 5'-TOP mRNAs are recruited to polysomes in a growth-dependent fashion that is selectively inhibited by rapamycin (72, 98). This finding alone does not distinguish between signaling through PHAS and signaling through p70S6k, because the pathway bifurcates downstream of mTOR (99) (see Fig. 2).

The insulin-stimulated pathways to general protein synthesis and to growth-regulated protein synthesis in 32D cells can be dissected with rapamycin (64). Insulin-stimulated total protein synthesis is inhibited only ~10%, and actin synthesis is not affected at all, but insulin-stimulated c-Myc synthesis is completely inhibited. Also, expression of constitutively active PKCζ in the absence of IRS-1, which bypasses the mTOR pathway, permits insulin-stimulated general protein synthesis but not c-Myc synthesis (23). This suggests that the insulin signal bifurcates at some site (23).

**Ca²⁺—**Ca²⁺ ions are involved in intracellular signaling and its distri-bution among compartments affect protein synthesis in at least two ways (26). Depletion of Ca²⁺ from the sarcoplasmic/endoplasmic reticulum by hormones like vasopressin inhibits initiation by phosphorylation of eIF2α via PKR (87). Elevation of cytosolic Ca²⁺ by agents such as bradykinin inhibits elongation by phosphorylation of eEF2 via eEF2K (26). Similarly, stimulation of the NMDA receptor causes eEF2 phosphorylation (88).

**Modification of Initiation Factors Can Affect the Spectrum of mRNAs Translated**

Signaling pathways do not stimulate translation of all mRNAs equally. Messenger RNAs differ widely in translational efficiency. Factors contributing to low efficiency of translation include a highly structured 5'-UTR, the presence of upstream AUGs, and poor sequence context for the initiating AUG (89), all of which are found in the 5'-UTRs of mRNAs for scarce proteins (90). mRNAs with these properties encode a disproportionate share of proteins involved in cell growth and cell cycle progression (89). These mRNAs are poorly translated in quiescent cells but preferentially recruited to ribosomes after a mitogenic signal (91–93). Overexpression of eIF4E in cultured cells preferentially stimulates translation of a number of mRNAs with high 5'-UTR secondary structure (89). Expression of cell cycle-dependent proteins like c-Myc (64) and cyclin D (94) requires mTOR, and inhibition of mTOR with rapamycin prolongs the G1 phase in both T-cells (95) and yeast (96). These results suggest that pathways that activate the unwind-ing machinery, i.e. by phosphorylation of eIF4E, eIF4G, or PHAS, disproporti-onately stimulate translation of growth-regulated mRNAs with high 5'-UTR secondary structure.

**Conclusions and Future Directions**

Protein synthesis is one of the most complicated biochemical processes undertaken by the cell, requiring roughly 150 different polypeptides and 70 different RNAs. Yet only seven polypeptides (eIF2α, eEF2B, eIF4E, eIF4G, S6, eEF1, and eEF2) have been identified as targets for regulatory pathways to date. Early observations that multiple initiation and elongation factors were phosphorylated in response to a single extracellular signal (101, 102) may have suggested unnecessary redundancy. This now seems more comprehensible when it is realized that modification of some factors affects the overall rate of translation whereas modification of others affects the spectrum of mRNAs translated. Understanding the pathways for regulation of protein synthesis holds promise for novel approaches for cancer intervention (e.g. Ref. 103), especially if pathways leading to growth-dependent protein synthesis can be selectively inhibited.

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