A Signaling Pathway to Translational Control

Minireview

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Over twenty years ago, an obligatory role for protein translation in G1 cell cycle progression was brought to light (reviewed by Norbury and Nurse, 1992). Transit through the cell cycle is thought to require a general increase in the rate of translation following growth factor-stimulation; in fact, even a partial inhibition of protein synthesis causes fibroblasts to accumulate in the G1 phase (Norbury and Nurse, 1992). It is now well known that growth factor stimulation causes a 2-3 fold mean increase in overall protein synthesis, implicating transmembrane signaling events in translational control (reviewed by Sonenberg, 1996). This average increase appears modest; however, while translation of some transcripts remains unaffected by mitogen stimulation, others increase greatly (discussed below). Although our understanding of the events that regulate gene expression through transcription has made steady progress, the signal transduction pathways involved in translational control have remained relatively uncharacterized. Catalyzed through use of cell-permeable inhibitors of cell cycle progression, one signaling pathway that leads to translational control has recently become better understood. This pathway is distinct from now well known ras/MAP kinase and Jak/Stat pathways that, among other outcomes, are involved in membrane-to-nuclear signaling events.

Rapamycin, initially characterized as an inhibitor of G1 cell cycle progression, has recently been used to illuminate a growth factor-regulated signaling pathway that leads to the enhanced translation of a specific subset of mRNAs. Translation of most transcripts is unaffected by the presence of rapamycin (Jefferies et al., 1994; Terada et al., 1994; Nielsen et al., 1995). In fact, treatment with rapamycin for 2-3 hours has only a slight inhibitory effect (15%) on overall protein synthesis (Jefferies et al., 1994; Terada et al., 1994; Mendez et al., 1996). However, translation of mRNAs derived from a few genes is inhibited significantly. The gene transcripts thus far identified as being rapamycin-sensitive include those encoding ribosomal proteins (S3, S6, S14, and S24), translation elongation factors (eEF1A and eEF2), and a secreted peptide growth factor called insulin-like growth factor II (IGF-II). As indicated by an increased association with multiple ribosomes (polyribosomes), these rapamycin-sensitive transcripts are translated at a 2-10 fold increased rate following mitogen stimulation (Jefferies et al., 1994; Terada et al., 1994; Nielsen et al., 1995). This shift to polyribosomes occurs with a concomitant decrease in the level of these mRNAs in ribonuclear protein (mRNP) particles, a putative storage facility for mRNAs. Rapamycin treatment inhibits each of these growth factor-stimulated effects (Jefferies et al., 1994; Terada et al., 1994; Nielsen et al., 1995). Since the change in association of these mRNAs with polyribosomes indicates that these transcripts are regulated at the initiation phase, the signaling pathway inhibited by rapamycin appears to regulate early steps in the translation process.

Regulation by the 5′ Untranslated Region

Translation initiation is generally the rate limiting step in the overall process and is influenced by elements within the 5′ untranslated region (UTR) of the mRNA. The rapamycin-sensitive translation of an IGF-II mRNA appears to be regulated by such a mechanism. Transcripts of the hormone IGF-II are alternatively spliced to produce multiple mRNAs that differ only in their 5′ UTRs. The translation of two such splice variants (4.8 and 6.0 kb) are discordant in their sensitivity to rapamycin treatment. While the 4.8 kb message is translated equally in rapamycin treated and untreated cells, translation of the 6.0 kb transcript is both rapamycin-sensitive and dependent on the presence of growth factors (Nielsen et al., 1995). Thus, the 1.2 kb leader sequence in the 5′ UTR of the 6.0 kb variant confers translational regulation to this gene.

What structural elements in 5′ UTRs may be involved in their translational regulation? One element implicated in the regulated translation of transcripts encoding ribosomal proteins and the eukaryotic elongation factors 1A and 2 (eEF1A and eEF2) is the polypyrimidine tract. This 5′ terminal oligopyrimidine tract (5′ TOP) typically consists of a stretch of 4-14 pyrimidines following the N^7-methylguanosine cap structure of the mRNA. Translational regulation of the ribosomal protein S16 transcript is abolished by replacement of five pyrimidines with purines in the 5′ UTR (Levy et al., 1991). A short stretch of pyrimidines is also found within 139 bp of the cap site of the 6.0 kb IGF-II transcript. Therefore, 5′ TOP tracts are candidate translational cis-regulatory elements, TLREs (Levy et al., 1991), that may be modulated by the rapamycin-sensitive signaling pathway.

However, there are other structural elements in the 5′ UTR of mRNAs that are known to influence translation initiation. It has been speculated that regulation of transcripts with long 5′ UTRs is due to the formation of secondary structures that must be overcome to provide efficient translation initiation (Sonenberg, 1996). In support of this hypothesis, sequences that form stable secondary structures ($\Delta G \leq -64$ kcal/mol) efficiently inhibit translation when inserted into the 5′ UTR (Sonenberg, 1996, and references therein). Examples of translationally-regulated mRNAs with long and complex 5′ UTRs include those that encode human FGF-5, c-myc and ornithine decarboxylase (Sonenberg, 1996). It is possible then that formation of stable secondary structures within the 5′ UTR of some transcripts, like that of IGF-II, may confer translational regulation that is dependent on rapamycin-sensitive signaling pathway. Such a hypothesis is supported by recent findings concerning the function of the eukaryotic initiation factor 4E (eIF4E).

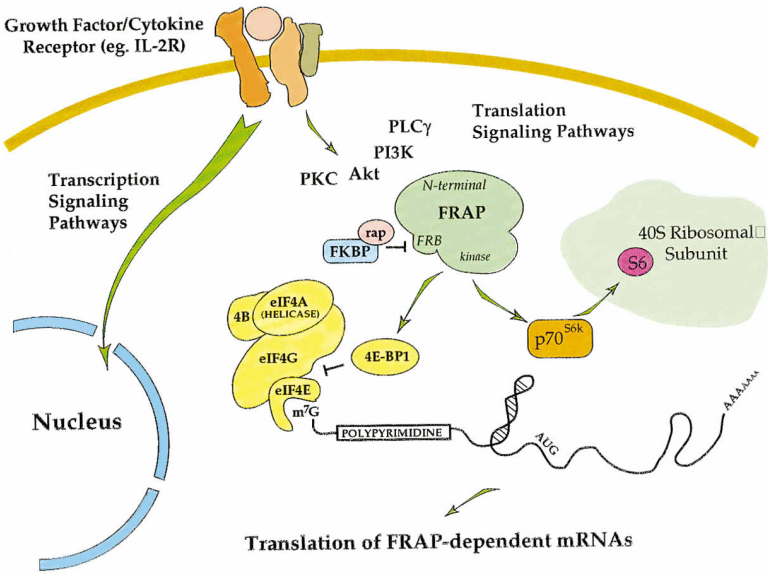

Figure 1. A Schematic Representation of the Growth Factor-Regulated Signaling Pathway that Leads to Translation Control

Transmembrane signaling events, involving the activation of phosphatidylinositol-3 kinase (PI3K) and phospholipase C-γ (PLCγ), mediate activation of p70 S6 kinase ($p70^{S6k}$) and phosphorylation of the eukaryotic initiation factor 4E (eIF4E) binding protein, 4E-BP1. Although not likely to be direct substrates for FRAP, both the activation of $p70^{S6k}$ by phosphorylation and the phosphorylation of 4E-BP1 are dependent on FRAP kinase activity, an activity inhibited by the FKBP12–rapamycin complex. Activated $p70^{S6k}$ phosphorylates the protein S6 in the 40S ribosomal subunit, an event that is correlated with growth factor-regulated increases in translation. Phosphorylation of 4E-BP1 prevents its inhibitory association with eIF4E. Translation initiation is catalyzed through the association of a complex of eIF4 initiation factors, including eIF4E and the RNA helicase eIF4A, with the N^7-methylguanosine (m^7G) cap on the mRNA. Transcripts that contain secondary structure in the 5′ untranslated region may be especially dependent on the association of the eIF4 complex.

Regulation of Translation Initiation Components

The initiation factor eIF4E is part of a complex of initiation factors (including eIF4A, eIF4B and eIF4G) that assemble on the mRNA cap site and possess an RNA helicase activity. The eIF4E binds directly to the N^7-methylguanosine cap structure of mRNA, providing a means for assembly of eIF4A, B, and G subunits on the 5′ end of mRNA (for further review see Sonenberg, 1996). Accumulation of these initiation factors on RNA is speculated to unwind secondary structures in the 5′ untranslated regions of mRNA and thereby allow efficient translation initiation (Sonenberg, 1996). Two phosphoproteins that are currently thought to be involved in the regulation of this complex of factors are the eIF4E binding proteins, 4E-BP1 and 4E-BP2. When in the dephosphorylated form, 4E-BPs bind directly to eIF4E and competitively inhibit association of eIF4G with eIF4E, an effect that is implied to prevent the assembly of the helicase complex on mRNA (for further review see Sonenberg, 1996). Growth factors are known to stimulate the phosphorylation of 4E-BP1 and cause its dissociation from eIF4E (Figure 1). Recently, two groups have reported that rapamycin potently inhibits the serum-induced phosphorylation of 4E-BP1 (Beretta et al., 1996 and references therein). Consistent with the inability of rapamycin to inhibit activation of MAP kinase and rsk, this phosphorylation is independent of the ras/MAP kinase pathway (von Manteuffel et al., 1996). Thus, the translational events that are dependent on the rapamycin-sensitive signaling pathway may be regulated in part through the phosphorylation of 4E-BPs.

However, 4E-BP1 is not the only connection between the rapamycin-sensitive signaling pathway and translational regulation. Another rapamycin-sensitive mediator of signaling pathways stimulated by growth factors is the serine/threonine kinase $p70^{S6k}$. Phosphorylation and activation of this kinase leads to the phosphorylation of the S6 protein in the 40S ribosomal subunit (reviewed by Chou and Blenis, 1995). Phosphorylation of S6 has long been known to correlate with the increased translation observed following mitogen stimulation. Moreover, ribosomes that contain a phosphorylated S6 protein are engaged in translation as polyribosomes a greater percentage of the time than ribosomes in which S6 is not phosphorylated. Rapamycin treatment causes the rapid inactivation of $p70^{S6k}$ and the dephosphorylation of S6 in vivo (reviewed by Chou and Blenis, 1995). However, since rapamycin does not affect translation generally but rather significantly inhibits only the translation of a subset of mRNAs, the role of S6 phosphorylation appears not to be a general requirement for translation. On these lines, it has been speculated that S6 phosphorylation is involved in the regulated translation of mRNAs that contain 5′ TOP elements (Jefferies et al., 1994; Terada et al., 1994).

Although together these studies can be constructed into attractive models, a great deal of research into the role of S6 and 4E-BP phosphorylation in translation regulation is still required. For example, it has been suggested that eIF4E is necessary for cap-dependent translation, a process that involves the translation of most eukaryotic mRNA. Furthermore, the relationship between secondary structure and 4E-BPs as well as that between 5′ TOP elements and S6 phosphorylation are preliminary models that are currently under research. It is not known whether regulation through these elements, or ones that have yet to be identified, may function in a rapamycin-sensitive pathway or whether the storage and removal of mRNAs from RNP particles may also play a role. Finally, it is possible that there are other rapamycin-sensitive factors that are involved in the translation of the rapamycin-sensitive mRNAs described earlier in this review. Nevertheless, the observed effects of rapamycin on both translation and on factors

such as 4EBP1 and p70^{S6k} describes a signaling pathway that emanates from growth factor receptors and leads to translational control. Since rapamycin is a specific inhibitor of this pathway, the molecule rapamycin inhibits must in some way regulate each of these growth factor-stimulated effects.

A Growth Factor Regulated Signaling Pathway for Translational Control

The inhibitory effects of rapamycin on cell cycle progression and translation are contingent first upon an association of rapamycin with the intracellular protein FKBP12 (reviewed by Chou and Blenis, 1995). It is now known that formation of this FKBP12–rapamycin complex provides a high affinity inhibitor of a 289 kd protein termed FRAP or RAFT1 (for *F*KBP12-*r*apamycin *a*ssociated *p*rotein or *r*apamycin and *F*KBP12 *t*arget, respectively). FRAP is a member of a newly discovered family of *p*hosphatidyl*i*nositol kinases-related kinases (PIK-related kinases) involved in events ranging from cell cycle regulation in response to stress to DNA recombination (reviewed by Keith and Schreiber, 1995). Although the biochemical regulation and function of FRAP is not completely understood, FRAP has an intrinsic protein kinase activity as evidenced by its ability to autophosphorylate on a serine residue in vitro (Brown, et al., 1995). Furthermore, FRAP has recently been shown to regulate p70^{S6k} activation in vivo in a manner that is dependent on both the kinase activity of FRAP and on an N-terminal domain (Brown et al., 1995). As implied by rapamycin-sensitivity, it is likely that 4E-BP1 phosphorylation is also regulated by FRAP. No interaction with or phosphorylation of p70^{S6k} or 4E-BP1 by FRAP has been observed, suggesting the existence of as yet unidentified components of this pathway. These studies suggest a role for FRAP in the pathway leading to the phosphorylation of both S6 and 4E-BP1 and to the regulated translation of a subset of mRNA transcripts.

Early signaling events that are involved in p70^{S6k} and 4E-BP1 regulation have been further elucidated through the use of a potent inhibitor of phosphatidylinositol-3-kinase (PI3K), wortmannin (Chou and Blenis, 1995). Treatment of cells with wortmannin prevents the growth factor-regulated increase in p70^{S6k} activity (Chou and Blenis, 1995) and 4E-BP1 phosphorylation (von Manteuffel et al., 1996). In addition, evidence in support of PI3K as an upstream regulator of p70^{S6k} and 4E-BP1 has been derived from studies using mutants of the PDGF receptor and of IRS-1, respectively (Mendez et al., 1996; reviewed by Chou and Blenis, 1995). Although it is not currently clear how the signal is propagated from PI3K to p70^{S6k} and 4E-BP1, FRAP does not appear to be upstream of PI3K (Chou and Blenis, 1995). In addition, phospholipase C-γ (PLCγ) and the protein kinases PKC and Akt have been implied to have some function in this pathway (Chou and Blenis, 1995). Thus, the outline of a growth factors-regulated signaling pathway that leads to translational control is beginning to emerge and is distinct from the now familiar MAP kinase pathway (Figure 1).

It is possible rapamycin's effects on translation are responsible for its ability to inhibit G1 cell cycle progression. In accord with such a hypothesis, the S. cerevisiae homologs of FRAP, TOR1, and TOR2, are now thought to regulate G1 progression through a translational mechanism (Barbet et al., 1996). Although little is known of how this regulation occurs, cell cycle progression in S. cerevisiae does not appear to require the phosphorylation of S6 since mutation of the phosphorylation sites in the yeast S6 protein (S10) does not hinder proliferation (Johnson and Warner, 1987). It may be that S6 phosphorylation functions differently in yeast than in mammals, since a heat shock stimulus has opposite effects on the phosphorylation state of S6 in these cells.

Recent studies have shown that regulated translation functions in modulating the activity of cyclin-dependent kinases in mammalian cells. It has recently been reported that the change in expression of the cyclin-dependent kinase inhibitor p27^{KIP1} following treatment with PDGF or lovastatin is mediated through an altered rate of translation of the encoding mRNA (Agrawal et al., 1996; Hengst and Reed, 1996). Since the mitogen-stimulated activity of cyclin-dependent kinases is sensitive to rapamycin treatment (Nourse et al., 1994, and references therein), it is possible that FRAP is on the pathway to the regulated translation of cyclin-dependent kinase inhibitors. Consistent with this model, treatment of T lymphocytes with interleukin-2 modulates the levels of the cyclin-dependent kinase inhibitors p21^{CIP1} and p27^{KIP1} in a rapamycin-sensitive fashion (Nourse et al., 1994). In the case of p21^{CIP1}, this regulation occurs without alteration of the levels of the encoding mRNA. It will be important to determine if the effect of rapamycin on p21^{CIP1} and p27^{KIP1} expression is through regulated translation, thus placing p21^{CIP1} and p27^{KIP1} among the other specific rapamycin-sensitive transcripts. Identification of the elements within 5′ UTRs of the rapamycin-sensitive transcripts and discovery of other intermediate molecules involved in this signaling pathway will be necessary to provide a complete link between growth factor signaling and the activation of cyclin-dependent kinases.

Feedback Regulation of Translation by Inhibitors of Translation Elongation

Growth factors however are not the only mechanism available to activate this rapamycin-sensitve signaling pathway. It has been known for over 20 years that small molecule inhibitors of protein translation, like cycloheximide and puromycin, also cause the phosphorylation of S6 in vivo (Gressner and Wool, 1974). More recently, anisomycin and cycloheximide treatment have been shown to activate p70^{S6k} in a rapamycin-sensitive fashion (references within Nielsen et al., 1995, and Chou and Blenis, 1995) and cause the phosphorylation of 4E-BP1 (von Manteuffel et al., 1996). Interestingly, treatment of chick embryo fibroblasts with cycloheximide at concentrations that only partially inhibit elongation causes an overall increase in the rate of synthesis of ribosomal proteins, some of the same proteins whose translation is activated by growth factors and inhibited by rapamycin (Ignotz et al., 1981). In addition, the small molecule anisomycin causes a 2-3 fold increase in the association of eEF1A and IGF-II (6.0 kb variant) mRNAs with polyribosomes (Nielsen et al., 1995). Overall, these results suggest that, in addition to a pathway initiated by growth factor receptors, an intracellular signaling cascade emanating from the protein synthesis machinery itself induces translational events that are rapamycin-sensitive.

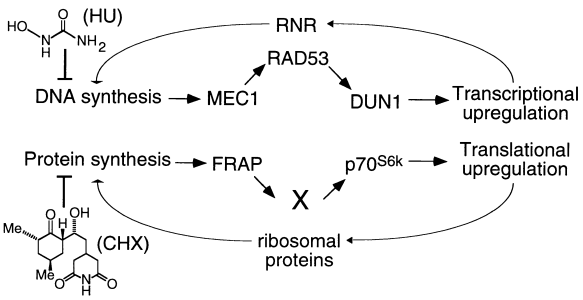

Figure 2. A Comparison of the Currently Understood Signaling Pathways Induced by Treatment with Inhibitors of DNA and Protein Synthesis

Hydroxyurea (HU) inhibits the activity of ribonucleotide reductase (RNR), an enzyme involved in the synthesis of deoxyribonucleotides. The transcriptional increase in RNR that occurs in response to HU treatment is dependent on the PIK-related kinase MEC1. Genetic studies indicate that the signals generated by MEC1 are mediated by two protein kinases, RAD53 and DUN1 (Sanchez et al., 1996). In analogy to the MEC1 pathway, inhibitors of 60S ribosomal subunit increase the translation of mRNA transcripts encoding ribosomal proteins and the eukaryotic elongation factor 1A (eEF1A). These inhibitors also induce the activity of $p70^{S6k}$ and the phosphorylation of 4E-BP1 in a manner that is dependent on FRAP. Arrows in this figure do not indicate direct interactions but rather illustrate the known sequence of events. Other molecules that have not yet been defined certainly may function in this pathway.

Other than FRAP, $p70^{S6k}$, and 4E-BP1, the molecules involved in this intracellular signaling pathway remain to be identified. Nevertheless, some clues may be obtained through an analysis of a signal transduction pathway propagated by another member of the PIK-related kinase family, MEC1.

MEC1 is an S. cerevisiae gene product that is homologous to FRAP and regulates the cell cycle progression in response to conditions that retard DNA synthesis, such as depletion of deoxyribonucleotides (Sanchez et al., 1996, and references therein). Other than regulating S phase progression, MEC1 responds to the depletion of deoxyribonucleotides (via hydroxyurea treatment) by mediating a signaling pathway that induces the expression of ribonucleotide reductase, an enzyme involved in the biosynthesis of deoxyribonucleic acids. This signaling pathway, which is just beginning to be understood, is strikingly similar to the activation of the FRAP/$p70^{S6k}$ pathway that is initiated by inhibitors of translation elongation (Figure 2). In analogy to the MEC1-mediated upregulation of ribonucleotide reductase in response to depletion of deoxyribonucleotides, FRAP appears to mediate an increase in the translation of ribosomal proteins in response to inhibition of the 60S ribosomal subunit. Thus, in addition to an involvement in growth factor–regulated events, these feedback pathways suggest a sensory role for FRAP analogous to that of MEC1. The implication of FRAP in this process and in the control of translation and cell cycle progression provides opportunities for further research in areas that are just beginning to emerge.

Selected Reading

Agrawal, D., Hauser, P., McPherson, F., Feng, D., Garcia, A., and Pledger, W.J. (1996). Mol. Cell Biol. *16*, 4327–4336.

Barbet, N.C., Schneider, U., Helliwell, S.B., Stanfield, I., Tuite, M.F., and Hall, M.N. (1996). Mol. Biol. Cell *7*, 25–42.

Beretta, L., Gingras, A.-C., Svitkin, Y.V., Hall, M., and Sonenberg, N. (1996). EMBO J. *15*, 658–664.

Brown, E.J., Beal, P.A., Keith, C.T., Chen, J., Shin, T.B., and Schreiber, S.L. (1995). Nature *377*, 441–446.

Chou, M.M., and Blenis, J. (1995). Cur. Opin. Cell Biol. *7*, 806–814.

Gressner, A.M., and Wool, I.G. (1974). Biochem. and Biophys. Res. Com. *60*, 1482–1490.

Hengst, L., and Reed, S. (1996). Science *271*, 1861–1864.

Ignotz, G.G., Hokari, S., DePhilip, R.M., Tsukada, K., and Lieberman, I. (1981). Biochemistry *20*, 2550–2558.

Jefferies, H.B.J., Reinhard, C., Kozma, S.C., and Thomas, G. (1994). Proc. Natl. Acad. Sci. USA *91*, 4441–4445.

Johnson, S.P., and Warner, J.R. (1987). Mol. Cell. Biol. *7*, 1338–1345.

Keith, C.T., and Schreiber, S.L. (1995). Science *270*, 50–51.

Levy, S., Avni, D., Hariharan, N., Perry, R.P., and Meyuhas, O. (1991). Proc. Natl. Acad. Sci. USA *88*, 3319–3323.

Mendez, R., Myers, M.G., White, M.F., and Rhoads, R.E. (1996). Mol. Cell Biol. *16*, 2857–2864.

Nielsen, F.C., Ostergaard, L., Nielson, J., and Christiansen, J. (1995). Nature *377*, 358–362.

Norbury, C., and Nurse, P. (1992). Annu. Rev. Biochem. *61*, 441–470.

Nourse, J., Firpo, E., Flanagan, W.M., Coats, S., Polyak, K., Lee, M.H., Massague, J., Crabtree, G.R., and Roberts, J.M. (1994). Nature *372*, 570–573.

Sanchez, Y., Desany, B.A., Jones, W.J., Liu, Q., Wang, B., and Elledge, S.J. (1996). Science *271*, 357–360.

Sonenberg, N. (1996). Translational Control, J.W.B. Hershey, M.B. Mathews, and N. Sonenberg eds. (Cold Spring Harbor Press), pp. 245–269.

Terada, N., Patel, H.R., Takase, K.Kohno, K., Nairn, A.C., and Gelfand, E.W. (1994). Proc. Natl. Acad. Sci. USA *91*, 11477–11481.

von Manteuffel, S.R., Gingras, A.-C., Sonenberg, N., and Thomas, G. (1996). Proc. Natl. Acad. Sci. USA *93*, 4076–4080.