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Regulation of signalling circuitry downstream of mTORC1(mammalian target of Rapamycin)

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ABSTRACT

The mammalian target of rapamycin (mTOR) is a 289-kDa serine-threonine kinase that integrates intracellular and extracellular cues to serve as a central and outsized regulator of organismal growth, cell metabolism, proliferation and survival. The pathway regulates crucial cellular processes and is implicated in a plethora of pathological conditions, including obesity, cancer, type 2 diabetes, and neurodegeneration. mTORC1 positively controls protein synthesis, required for cell growth, via various downstream effectors like ribosomal protein S6 kinase and eIF4E binding proteins 4E-BPs. Here, we review the regulation of mTOR substrates, their phosphorylation dynamics, binding partners, and modes of activation. We also discuss role of mTOR pathway targets in health, disease as well as cancer. We further discuss pharmacological strategies to treat human pathologies linked to mTOR deregulation.

Keywords: Intracellular, Kinase, Metabolism, Phosphorylation, Rapamycin.

I INTRODUCTION

The mammalian target of rapamycin (mTOR) is a conserved serine theronine kinase belonging to the phospho-inositide 3-kinase (PI3K)-related kinase family. It was first discovered in yeast *Saccharomyces cerevisiae* during the screen for resistance to the immunosuppressant drug rapamycin. mTOR nucleates at least two distinct multi-protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The two complexes contain multiple components, most of which are similar. mTORC1 consists of five components which include mTOR, the catalytic subunit of the complex; regulatory-associated protein of mTOR (Raptor); mammalian lethal with Sec13 protein 8 (mLST8, also known as GβL); proline-rich AKT substrate 40 kDa (PRAS40); and DEP-domain-containing mTOR-interacting protein (Deptor). The mTORC2 complex includes mTOR; rapamycin-insensitive companion of mTOR (Rictor); mammalian stress-activated protein kinase interacting protein (mSIN1); protein observed with Rictor-1 (Protor-1); mLST8; and Deptor [1].mTORC1 is the master regulator of cellular growth, plays role in protein synthesis, lipid synthesis, autophagy and mitochondrial metabolism and biogenesis. It is activated by different environmental cues which include growth factors, insulin, cytokines, nutrients, and cell stress. The activation of mTORC1 is mediated through insulin/PI3K pathway. The binding of insulin or IGF to its cognate cell surface receptor results in the phosphorylation of insulin receptor

substrate (IRS) proteins at their tyrosine residue resulting in the recruitment and activation of PI3K. Activated PI3K in turn generates PtdIns(3,4,5)P3 which in turn leads PDK1 mediated phosphorylation of AKT on its T-loop site (Thr308). The activation of AKT indirectly increases signaling of mTORC1 by phosphorylating Tsc2 on several sites (Ser939 and Thr1462) and thereby suppressing the inhibitory effect of Tsc1–Tsc2 complex on mTORC1. The mTORC1 mediates its downstream effects through its well-defined targets which include ribosomal protein S6 kinase, and 4E binding protein 4E-BP1. The fungal macrolide rapamycin abolishes the activity of mTORC1, resulting in the reduction of phosphorylation of S6Kinase and 4E-BP1 [1].

II REGULATION AND PHOSPHORYLATION DYNAMICS OF S6KINASE

S6K1 (p70/p85) is a major downstream effector of mTOR. mTORC1 has been shown to directly phosphorylate S6K1 as revealed by the macrolide substance rapamycin a known inhibitor of mTOR, that potentially inhibits mitogen induced activation of S6K1 and consequently the phosphorylation of ribosomal protein S6 [2, 3, 4]. S6K1 structure–function studies have elucidated the molecular steps that govern S6K1 activation by mitogens, revealing roles for complex interactions between specific domains and phosphorylation sites. The activation of S6K1 is brought about by increased phosphorylation at eight residues [5] among which three appear to be essential in regulating kinase activation. These include Thr229 in the catalytic domain, Ser371 and Thr389 in the linker domain [5,6]. A variety of kinases that function upstream of S6K1 have been found responsible for the phosphorylation of S6K1 resulting in its activation. These include phosphoinositide-3-kinase (PI3K), mammalian target of rapamycin (mTOR), and PDK1. The PI3K (phosphoinositide 3-kinase)/Akt induces its effect on S6K1 by activating mTORC1 upon insulin/IGF stimulation and thus the S6Ks [7]. The Ras/MAPK (mitogen-activated protein kinase) also activates mTORC1 in a PI3K-independent manner. Both pathways, however, co-operate with other inputs to maximally activate the S6Ks. The Rho family of G-proteins which include Cdc42 and Rac and the atypical PKC isoforms PKCζand PKCλ that function downstream of PI3Kcontribute S6K1 activation [8,9]. However, the downstream effector linking PI3K with p70S6K has not been identified [10]. The mTOR mammalian target of rapamycin also called (FRAP/RAFT/FKBP12) has been shown to directly phosphorylate S6K1 on the HM site Thr389 to promote S6K1 activity [11,12]. Mutation of Thr389 to alanine (T389A) abolishes S6K1 activity, whereas substitution of an acidic glutamate residue for Thr389 (T389E) to mimic phosphorylation augments basal S6K1 activity in the absence of mitogens, thus rendering S6K1 partially constitutively active [13,14,15]. The serum-stimulated activation and the phosphorylation of the rapamycin-sensitive sites Thr389, Thr229 and Ser404 of S6K1 has been shown to be abolished by deletion of 30 amino acids from the N-terminus of p70-S6K1 (ΔNT) and the phosphorylation of the rapamycin-sensitive sites Thr389, Thr229 and Ser404 [15,16,17]. Strikingly, additional deletion of the C-terminus (ΔNT/ΔCT) results in the restoration of kinase activity (although to levels significantly less than maximal, 5–15%) and phosphorylation of the rapamycin-sensitive sites. These results indicated that the N-terminus of S6K1 functions in the reception of an activating input critical for Thr389 and Thr229 phosphorylation and suppresses an inhibitory function mediated by the C-terminus. A short sequence of 5 amino

acids (FDIDL), at the N-terminal region of S6K1 named the TOS motif has been identified to be critical for mitogen-stimulated S6K1 activation and phosphorylation of rapamycin-sensitive sites [14]. The deletion or mutagenic inactivation of the motif (F5A mutation within the FDIDL sequence) of TOS motif abolishes S6K1 kinase activity as well as Thr389 and Thr229 phosphorylation, thus mapping the critical regulatory function of the N-terminus to a specific motif. As in ΔNT/ΔCT, deletion of the C-terminus from the F5A mutant (F5A-ΔCT) partially restored kinase activity and Thr389 phosphorylation. There is alsothe presence of RSPRR motif in the C-terminus of p70-S6K1 (amino acids 410–414), shortly after the linker region. PDK1 has been found to selectively phosphorylate p70S6K at Thr229 in vitro and in vivo upon mitogen stimulation using Myc-tagged PDK1 and Myc-p70S6K expressed in 293 cells, whereas the catalytically inactive PDK1 blocks insulin-induced activation of p70S6K [18]. The phosphorylation of Thr389 is a prerequisite for phosphorylation of Thr229which is facilitated by converting four (Ser/Thr)-Pro phosphorylation sites in the autoinhibitory domain to acidic residues [19]. The stepwise activation of S6K1 via ordered multi-site phosphorylation revealed strong positive co-operativity between phosphorylation of Thr229 and Thr389.

III ACTIVATION OF S6KINASE AND EFFECTS OF IMPAIRED LEVELS THEREOF

The activation of S6K1 occurs by diverse stimuli through a multisite phosphorylation directed at three separate domains as follows: a cluster of (Ser/Thr) Pro sites in an autoinhibitory segment in the noncatalytic carboxyl-terminal tail; Thr-252 in the activation loop of the catalytic domain; and Ser-394 and Thr-412 in a segment immediately carboxyl-terminal to the catalytic domain. Phosphorylation of Thr-252 in vitroby the enzyme phosphatidylinositol 3-phosphate-dependent kinase-1 or mutation of Thr-412 to Glu has each been shown to engender some activation of the p70 S6 kinase, whereas both modifications together produce 20–30-fold more activity than either alone. The use of phospho-specific anti-peptide antibodies has shown substantial phosphorylation of p70 Thr-252 and Ser-434 in serum-deprived cells, whereas Thr-412 and Thr-444/Ser-447 were essentially devoid of phospho-specific immunoreactivity. Activation of p70 by insulin was accompanied by a coordinate increase in phosphorylation at all sites examined, together with a slow mobility on SDS-PAGE of a portion of p70 polypeptides.S6K1 has also been reported to get activated in response to viral infection; such that baculovirus mediated expression of the enzyme in insect cells activates the enzyme by phosphorylation at similar sites as identified in the enzyme from regulated cells[20]. Since it stands established that insect S6 kinase, behaves similar to that of its mammalian counterpart, it is conceivable that the activation state of the enzyme and its inhibition by rapamycin would be no different than the one established for mammalian systems [21,22].

In addition to its function as a major regulator of translation and glucose homeostasis, S6K1 is also responsible for the development of normal body phenotype. Studies have shown that deletion of S6K1 in drosophila is semi lethal, with few surviving individuals that have a severely reduced body size. That is due to a decrease in cell size rather than a decrease in cell number. The larvae of such flies exhibit a long developmental delay, consistent with a two-fold increase in cell cycle doubling times and the surviving adults are quite lethargic,

living no longer than 2 weeks and females are sterile[23]. Mice with deleted S6K1 show similar phenotype as in drosophila. Mice with removed S6K1 were born smaller but not lethal. However, S6k1 deficient mice have hypoinsulinemia, glucose intolerance and diminished β-cell size [24,25]. S6K1 has also been implicated in the Fragile X syndrome (FXS), the leading inherited cause of autism and intellectual disability. The dysregulated protein synthesis is the key causal factor in FXS. It has been shown that S6K1 deletion resulted in the prevention of elevated phosphorylation of translational control molecules, exaggerated protein synthesis, and enhanced mGluR-dependent longterm depression (LTD), weight gain and macrochidism, immature dendritic spine morphology and multiple behavioural phenotypes, including social interaction deficits, impaired novel object recognition and behavioural inflexibility characteristic of FXS syndrome in FXS model mice. In addition, it has been found that*Fmr1*/*S6K1* double knockout (dKO) mice resulted in the normalization of enhanced mGluR-LTD) [26]. Genetic reduction of *S6K1* may therefore serve as a potential target for therapeutic intervention in humans with FXS.

IV S6K1 AND CANCER

S6K1 has been reported to over express in certain cancers, particularly in breast cancer due to 17q23 amplification of RPS6KB1 gene and this phenotype correlates with poor prognosis [27]. S6K1 has been suggested to contribute tumorigenesis in tissues bearing low levels of S6K2) [28] P70S6 kinase is frequently activated in human ovarian cancer. There is significantly more constitutive activation of P70S6K in malignant tumors than in benign or borderline lesions [29]. The growth factors including EGF and HGF also activate P70S6K and act as potent inducers of P70S6K in ovarian cancer cell lines [30,31]. P70S6K has been shown to function in epithelial to mesenchymal transition (EMT) responsible for the acquisition of invasiveness during tumor progression. The ability of P70S6K to repress E-Cadherin through the upregulation of snail is responsible for its tumorogenic activity. The activation of P70 induced phenotypic changes consistent with EMT in ovarian cancer cell lines. Western blot analysis has shown that decreased expression of epithelial marker E-Cadherin and increased expression of mesenchymal markers N-Cadherin and vimentin. Inhibition of p70S6K by a specific inhibitor or small interfering RNA reversed the shift of EMT markers. The induction of p70S6K in EMT is Snail specific, as knockdown of Snail results in the suppression of p70S6K [32]. These results indicate that p70 may play a critical role in tumor progression in ovarian cancer through the induction of EMT. Targeting P70S6K may thus be a useful strategy to impede cancer cell invasion and metastasis. S6K1 yields a long active kinase p85/p70S6K1 (referred to as Iso-1) splice variant and shorter splicing variants (referred to as Iso-2) in mouse and h6A and h6C in humans. Studies have shown that SRSF1 results in the increased expression of the shorter S6K1 isoform which possesses the oncogenic activity and can transform immortal mouse fibroblasts [33]. Another study has also showed that short kinase inactive isoforms posess oncogenic properties while as Iso1 form is tumor suppressive invitro and invivo and can block Ras-induced transformation [34]. Therefore, S6K1 and its isoforms may provide a potential therapeutic target for cancer therapy.

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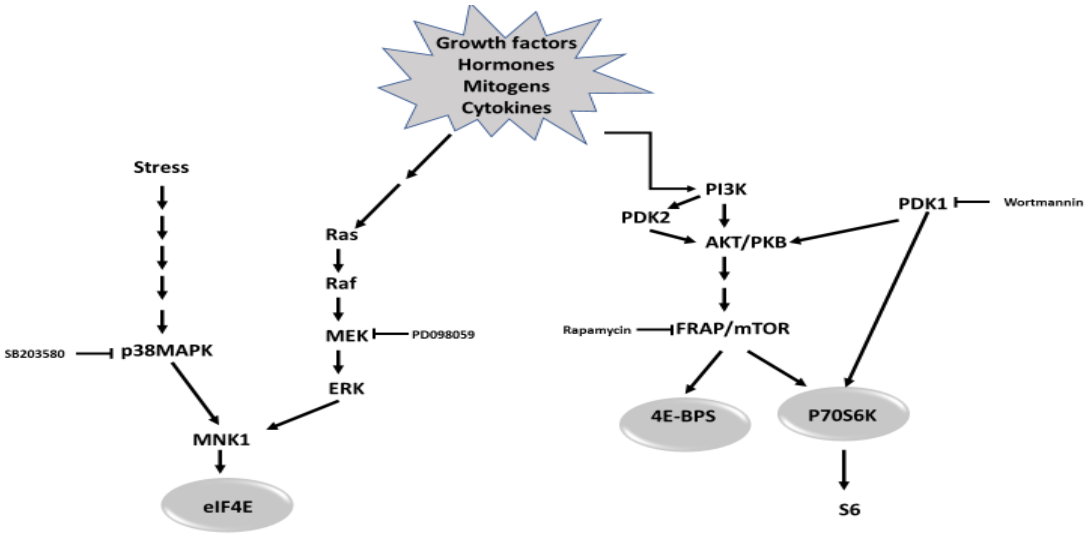

Figure 1. The intracellular signalling pathways impinging upon the translation initiation factors eIF4E and the 4E-BPs. The Ras pathway, leading to eIF4E phosphorylation, and the PI3K pathway, leading to 4E-BP and p70S6k phosphorylation, are depicted. Also shown are the targets of several of the pharmacological inhibitors utilized in the studies delineating these pathways.

V REGULATION OF eIF4E ACTIVITY

Recruitment of the translational machinery to mRNA is responsive to a variety of extracellular stimuli, including exposure to hormones, growth factors and cytokines, nutrient availability, and various types of cellular stresse[35]. eIF4E activity is regulated at multiple levels; (1) via modulation of its transcription, (2) by phosphorylation of the eIF4E protein, and (3) through its interaction with a family of translational repressor proteins.

5.1 Transcriptional regulation of eIF4E

eIF4E mRNA expression is increased several-fold in fibroblasts in response to serum or growth factor treatment [36] and increases rapidly upon activation of human T cells [37]. The eIF4E promoter contain two bona fide myc binding sites, both of which are required for expression of a heterologous reporter gene [38]. eIF4E mRNA expression is upregulated in cells overexpressing c-myc, and transcription of the eIF4E gene is responsive to activation of a myc-estrogen receptor fusion protein [36]. Myc protein is a potent regulator of cell proliferation [39]. Since eIF4E itself apparently plays a key role in cell growth and proliferation, it is possible that eIF4E is an important downstream target of myc.

5.2 eIF4E Phosphorylation Dynamics

A second level of eIF4E regulation is phosphorylation. The phosphorylation state of eIF4E is, in general, correlated with the translation rate and growth status of the cell [40,41]. For instance, eIF4E is

underphosphorylated in mitosis [42], a phase in which translation, rates are low. eIF4E phosphorylation is influenced by a variety of extracellular stimuli: treatment of cells in culture with hormones, growth factors, cytokines or mitogens results in a net increase in eIF4E phosphorylation. An increase in eIF4E phosphorylation is also correlated with an increased cardiac load [43] and angiotensin II, a hypertrophic factor, induces eIF4E phosphorylation in smooth muscle cells [44] The correlation between eIF4E phosphorylation and the overall translation rate is, however, not observed in every situation. For example, an increase in eIF4E phosphorylation is observed in response to some types of cellular stress, including exposure to anisomycin, arsenite ,tumor necrosis factor-α and interleukin-1β [45,46] even though translation rates actually decrease in these situations. However, other types of cellular stress, including heat-shock [47], or infection with adenovirus or encephalomyocarditis virus (EMCV) are accompanied by a decrease in eIF4E phosphorylation [48]. eIF4E phosphorylation has been reported to increase its affnity for mRNA caps [49], and some credence is added to this observation with the determination of the three-dimensional structure of eIF4E. The phosphorylation of mammalian eIF4E in response to all stimuli so far examined occurs on Ser209 (numbering for the murine protein), with some minor phosphorylation detected in certain cases on threonine residues (most likely Thr210). Although protein kinase C (PKC) can efficiently phosphorylate eIF4E in vitro on Ser209 [50],the precise role of PKC in the phosphorylation of eIF4E in vivo remains unclear.PKC plays an important role in mediating eIF4E phosphorylation [51]. A putative role for the ras/raf/MAPK(ERK) pathway [52,53] in eIF4E phosphorylation was suggested by reports demonstrating an increased phosphorylation of eIF4E in ras- or src-transformed cells [54,55].The ERK signalling cascade is activated following growth factor, hormone, cytokine or mitogen treatment, and is inhibited specifically by the drug PD098059, which prevents MAP kinase kinase (also known as MEK; MAPK/ERK kinase) activation [56]. Phosphorylation of eIF4E induced by serum or insulin is prevented to a great extent by simultaneous treatment with PD098059 [57,58]. However, as direct role for the MAPKs in eIF4E phosphorylation, the ERKs cannot phosphorylate eIF4E in vitro [58]. The p38 subfamily of MAPKs, like the JNK family of MAPKs, is activated by several types of cellular stress, including hyperosmolality, heat shock, UV irradiation, or exposure to LPS, arsenite, or anisomycin. Although not as well characterized as the ERK activation cascade, activation of p38 MAPKs also involves a phosphorylation cascade [53,59]. p38 MAPK activity (but not JNK activity) is specifically prevented by the pharmacological compound SB203580 [60] and phosphorylation by anisomycin is prevented in cells preincubated with SB203580 [46,57] .It has been demonstrated that the mitogen-stimulated pathway acting through the ERKs, and the stress-activated pathway acting through the p38 MAPKs, appear to converge at a common eIF4E kinase called MNK1 (for MAP kinase interacting kinase SSSSS1, or MAP kinase signal integrating kinase 1). MNK1 was isolated via interaction screening as a substrate for both ERKI and p38MAPK, and activation of either ERKI or p38MAPK (but not the JNK kinases) stimulates MNK1 kinase activity [61,62]. MNK1 efficiently phosphorylates eIF4E in vitro on Ser209 [62]and thus appears to be a likely candidate to phosphorylate eIF4E in vivo, following stimulation of either the ERK or p38 MAPK cascades.

5.3 4E-BPS as regulators of eIF4E

A third level of eIF4E regulation is through its interaction with a family of inhibitory binding proteins. Using the Far-Western hybridization technique [63] isolated cDNAs encoding two small (12 kDa) proteins which interact with eIF4E, and which share 56% overall identity at the amino acid level. These proteins, termed 4E-BP1 and 4E-BP2 (for eIF4E-binding proteins 1 and 2) were demonstrated to inhibit cap-dependent translation, both in an in vitro cell-free translation assay, and in vivo [63].Cap-independent translation is not affected by the 4E-BPs. Binding of the 4E-BPs to eIF4E does not alter the affinity of eIF4E for the cap structure [63]; rather, it prevents the association between eIF4G and eIF4E and, thus, the assembly of a functional eIF4F complex[64]. Both eIF4G and the 4E-BPs share a small amino acid motif (YXXXXLF, where X is any amino acid, and F is an aliphatic residue) responsible for interaction with eIF4E [65]. Deletion of this sequence or mutation of either the tyrosine or the LF residue(s) to alanine(s) abolishes eIF4E binding [65]. Further establishing the importance of this binding motif, a 20mer containing this sequence significantly inhibits cap-dependent translation in an in vitro translation assay [66]. NMR and crystallographic data also confirmed the importance of this 4E-BP1 segment in mediating binding to eIF4E [67]. A third member of the 4E-BP family, 4E-BP3, has been cloned [68]. 4E-BP3 shares a high degree of homology with 4E-BP1 and 4E-BP2 in the middle region of the protein, which contains the eIF4E-binding site [63], and, as expected, 4E-BP3 is inhibitory to cap-dependent, but not cap-independent translation.

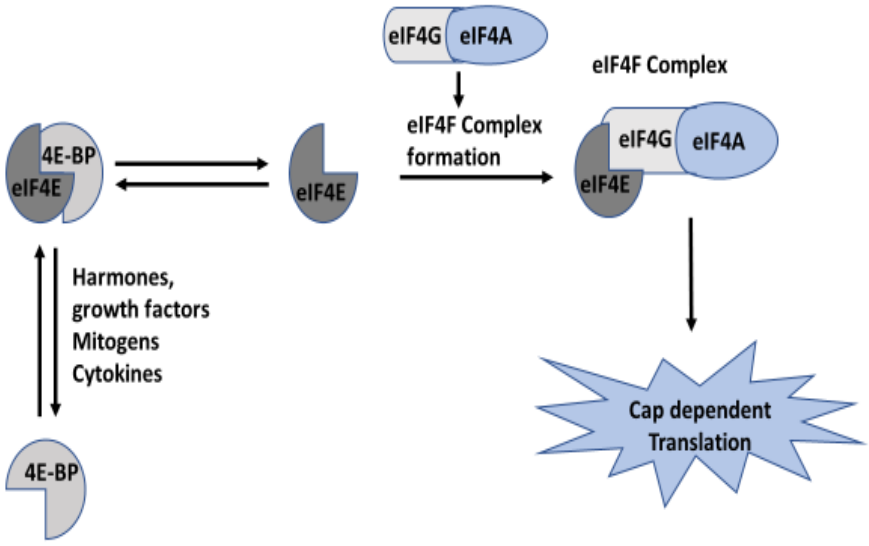

Fig. 2. Regulation of the formation of the eIF4F cap binding complex.

A structural homologue of the mammalian 4E-BPs does not exist in Saccharomyces cerevisiae, whose genome is completely sequenced. However, an apparent functional 4E-BP homologue, designated p20, has been isolated [68]. p20 does not share sequence homology with the 4E-BPs, except for the presence of an eIF4E-binding motif (YXXXXLL) similar to that of the mammalian eIF4E-interacting proteins. In an in vitro translation-competent yeast extract, p20 inhibits cap-dependent, but not cap-independent translation. Also, similar to the

mammalian 4E-BPs, p20 competes with the yeast eIF4G homologues for binding to eIF4E. Overexpression of p20 in yeast cells results in slower growth, whereas disruption of the gene encoding p20 stimulates growth of yeast in culture [68]. Thus, attesting to the importance of this mode of translational control, it appears that eIF4E regulation via an inhibition of its interaction with eIF4G evolved independently in yeast and mammals.

VI REGULATION AND PHOSPHORYLATION DYNAMICS OF 4E-BPs

The phosphorylation state of specific serine/threonine residues in the 4E-BPs regulates the affinity of these proteins for eIF4E [63,69,70]. Hypophosphorylated 4E-BPs bind efficiently to eIF4E, but phosphorylation of a critical number of residues in 4E-BP1 abrogates this binding. Like eIF4E, 4E-BP phosphorylation levels are influenced by many types of extracellular stimuli. In this regard, 4E-BP1 was independently cloned as a major phosphorylation substrate following insulin stimulation of rat adipocytes, and is also known as PHAS-I(phosphorylated heat and acid stable insulin responsive protein) [70]. Hormones (insulin, angiotensin), growth factors (EGF, PDGF, NGF,IGFI, IGFII), cytokines (IL-3, GMCSF + steel factor), mitogens (TPA), G-protein coupled receptor ligands (gastrin), and adenovirus infection have all been reported to induce phosphorylation of 4E-BP1, accompanied by a resultant decrease in its ability to interact with eIF4E [51,71,72]. Conversely, heat shock (in certain cell types) [73] and infection with poliovirus or EMCV [74] have been reported to decrease 4E-BP1 phosphorylation. In the case of EMCV infection, the time course of 4E-BP1 dephosphorylation correlates with the time course of host cell translation shut-off.

6.1 Identification of upstream kinases in the 4E-BP inactivation pathway

Phosphorylation of the 4E-BPs does not appear to be regulated through the ras/raf/ MAPK pathway. MAPK/ERK activation is not necessary in 293 and Swiss 3T3 cells for induction of 4E-BP1 phosphorylation: insulin, in these cells, does not activate MAPK, yet induces hyperphosphorylation of 4E-BP1 [75]. Further,the MEK inhibitor PD098059 does not prevent 4E-BP1 phosphorylation in response to various stimuli [71,76,77]. However, inhibitors which are without effect on MAPK activity (rapamycin,wortmannin, and SQ20006) completely prevent 4E-BP1 phosphorylation [78,79] . These data are in contrast to initial reports, based mostly on in vitro studies, suggesting that the ERKs were the kinases responsible for 4E-BP1 phosphorylation[74]. Interestingly, the intracellular signalling cascade leading to 4E-BP1 phosphorylation shares many similarities with the pathway leading to p70S6k activation (p70S6k is the protein kinase responsible for the phosphorylation of the ribosomal protein S6). 4E-BP1 phosphorylation is also dependent upon the FKBP-rapamycin associated protein/mammalian target of rapamycin (FRAP/mTOR) kinase [80,81].The PI3Ks are a family of lipid kinases responsible for the phosphorylation of the hydroxyl group at position three of the inositol ring of phosphatidylinositols. PI3Ks have been implicated in the regulation of many cellular processes, including resistance to apoptosis, cell motility,differentiation and proliferation [82].Wortmannin inhibits PI3K signalling by covalently binding to its catalytic subunit [83]. Overexpression of the PI3K a catalytic subunit (p110 α) induces phosphorylation of 4E-BP1 in a wortmannin-sensitive manner [79].The Akt/PKB Ser/Thr protein

kinases were first identifed as cellular counterparts of the transforming viral oncogene v-akt [84]. Akt was demonstrated to inhibit apoptosis in several cell types, at least in part, by phosphorylating and inactivating the apoptotic inducer BAD [85] .Akt is activated by PI3K-generated lipid products, which bind to its pleckstrin homology (PH) domain, and target Akt to the plasma membrane [86]. This translocation apparently enables the subsequent phosphorylation of Thr308 (located in the kinase domain of Akt) by the kinase PDK1. PDK1 is neither stimulated by insulin nor inhibited by PI3K inhibitors, and has been described to also phosphorylate a site in p70S6k which is crucial for activation, and which resembles the Akt Thr308 site [18]. To fully activate Akt, phosphorylation of Ser473, mediated by PDK2, must also be accomplished. PDK2 kinase appears to also be activated by the lipid products of PI3 kinase [86].A constitutively active, membrane-targeted form of Akt can be engineered by introducing a srcmyristoylation signal in its amino-terminus (MyrAkt). Cells expressing MyrAkt survive treatment with apoptosis-inducing agents or growth factor deprivation [83,87] Overexpression ofAkt also induces 4E-BP1 phosphorylation on the same sites observed to be phosphorylated in response to serum stimulation [79]. The requirement for Akt in mediating 4E-BP1 phosphorylation is suggested by the fact that overexpression of a dominant-negative form of Akt prevents the increase in 4E-BP1 phosphorylation observed following insulin stimulation [79]. FRAP/mTOR is the mammalian homologue of the yeast TOR proteins, and the target of the FKBP12-rapamycin complex (an immunophilin-immunosuppressant interaction). This very large (289 kDa) protein is a member (together with ATM, ATR/FRP and DNA-PK) of a newly emerging family of kinases, termed the PIKs (phosphatidylinositol kinase-related kinases) [88,89].Although initially identified via their homology to lipid kinases (and especially to phosphoinositide-3 kinases), some members of this family appear to function, instead, as protein kinases[88,89].The role of FRAP/mTOR in mammalian translation initiation was confirmed when it was demonstrated that expression of a rapamycin-resistant FRAP/mTOR protein confers rapamycin resistance to 4E-BP1 phosphorylation [80,81]. Immuno-precipitates of FRAP/mTOR were also reported to phosphorylate 4E-BP1 in vitro.p70S6k, through an unknown mechanism, regulates the translation of a specific subset of mRNAs [90].This kinase does not signal upstream of 4E-BP1, but lies on a parallel pathway and appears to share a common upstream activator with 4E-BP1 (Fig. 4). This conclusion was reached after demonstrating that a rapamycin-resistant form of p70S6k was unable to confer rapamycin-resistance to 4E-BP1. Rather, overexpression of the rapamycin-resistant p70S6k, as well as overexpression of any other active or inactive p70S6k protein, leads to a decrease in 4E-BP1 phosphorylation [78]. This result suggests that p70S6k overexpression titters out (or squelches) an upstream kinase involved in 4E-BP1 phosphorylation.

VII eIF4E and 4E-BP1 IN CANCER INITIATION AND PROGRESSION

The oncogenic potential of eIF4E hyperactivity has been well described both in vitro and in vivo. Overexpression of eIF4E is sufficient to induce transformation of fibroblasts and primary epithelial cells [91,92]. eIF4E overexpression in vivo leads to increased cancer susceptibility; eIF4E transgenic mice develop lymphomas, angiosarcomas, lung carcinomas and hepatomas [93]. Additionally, eIF4E overexpression can

overcome Myc-induced apoptosis, which demonstrates that eIF4E possesses intrinsic oncogenic activity that can overcome the cellular barrier of apoptosis resulting from Myc induced oncogenic stress [93,94]. eIF4E gene amplification has been reported in human breast cancer and in head and neck cancer specimens compared to noncancerous control samples [95].

While an increase in cellular eIF4E activity is in itself oncogenic, common signaling pathways that are heavily mutated or amplified in human cancers directly impact eIF4E activity as well. For instance, the eIF4E promoter contains two E box domains that are direct targets of c-myc. c-myc overexpression drives eIF4E transcription, while dominant negative myc represses eIF4E transcription [96]. It was demonstrated that in the setting of oncogenic activation of the PI3K/AKT/mTOR signaling pathway, phosphorylation of 4EBP1 resulting in hyperactivation of eIF4E is required for lymphomagenesis as well as cancer progression in vivo. Interestingly, inhibition of phosphorylation of ribosomal protein S6 (rpS6) (another downstream arm of mTOR) by mTOR does not decrease the rate of tumorigenesis and thus supports the primary role of the 4EBP1/eIF4E axis in cancer formation downstream of oncogenic mTOR [97]. Activation of the oncogenic RAS (an upstream regulator of the MNK kinase) and AKT signaling pathways induce the formation of glioblastoma multiforme (GBM) in neural progenitor cells, which is accompanied by a dramatic upregulation in translational regulation of oncogenic networks [98]. However,is unknown whether eIF4E hyperactivation plays a direct role downstream of oncogenic MYC and RAS towards cancer progression. Downstream oncogenic AKT, it was found that blocking eIF4E hyperactivity after tumor formation in vivo results in inhibition of tumor growth [97].Taken together, eIF4E is an oncogene as well as an important target for oncogenic translational deregulation in the context of MYC,PI3K/AKT/mTOR and RAS overexpression. Therefore, eIF4E represents an attractive therapeutic target in human cancers as it embodies an integrating focal point downstream of these bona fide oncogenic pathways.

VIII CONCLUDING REMARKS

Work over the last decade has reported a rapid increase in interest and knowledge about the mTOR pathway. Activation of mTORC1 results in the downstream phosphorylation of two main effectors: eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1). We highlight the complex relationship with upstream regulators of mTOR and the downstream targets of mTOR activation.Much surely remains to be discovered, but we discuss enough about the pathway and its function and regulation in normal and disease physiology which can be carried forward for therapeutic benefit.

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