Oncogene (2004) 23, 3138–3144

www.nature.com/onc

Signaling control of mRNA translation in cancer pathogenesis

Eric C Holland*,[1], Nahum Sonenberg[2], Pier Paolo Pandolfi[3] and George Thomas[4]

[1]*Departments of Surgery, Neurology and Cell Biology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA;* [2]*Department of Biochemistry, McGill University, 3655 Promenade Sire William Osler, Montreal, Canada;* [3]*Molecular Biology Program and Dept. of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA;* [4]*Friedrich-Miescher Institute for Biomedical Research, Maulbeerstrasse 66, Basel CH4058, Switzerland*

The regulation of translation and the control of ribosome biogenesis are essential cellular processes whose impact on cell growth and proliferation is manifested at a number of specific levels. Disruption in one or more of the steps that control protein biosynthesis has been associated with alterations in the regulation of cell growth and cell cycle progression. Consistent with this, tumor suppressors and proto-oncogenes have been found to act on these functions and may therefore regulate malignant progression by affecting the protein synthetic machinery. Although many studies have correlated deregulation of protein biosynthesis with cancer, it remains to be established whether this process is necessary and/or sufficient for neoplastic transformation and metastasis.
Oncogene (2004) **23,** 3138–3144. doi:10.1038/sj.onc.1207590

Keywords: mRNA translation; PTEN; TSC1/2; mTOR; Rheb; eIF4E; prostate cancer; glioblastoma

Ras, an important effector in translation control

The effects of growth factors and hormones (e.g. insulin) on the protein synthetic machinery are mediated by signal transduction cascades, which in turn modulate the specific activity of key translation factors (Figure 1). Moreover, growth factors stimulate global protein synthesis by increasing both the rates of translation initiation and elongation as well as by triggering ribosome biogenesis to increase translational capacity (Antonetti *et al*., 1993). The activation of growth factor signal transduction pathways is initiated by the binding of the ligand to their tyrosine kinase receptors, leading to the activation of the Ras and PI-3K signaling pathways. Ras signaling activates MAP kinase, specifically Erk1/2, which in turn activates MAP kinase-activated protein kinase (Mnk)1 and 2, two kinases that mediate eukaryotic initiation factor eIF4E-Ser 209 phosphorylation (Scheper *et al*., 2001) (Figure 1), an apparent critical event in driving increased translation. Consistent with this, increased eIF4E expression alone can transform cells both *in vitro* and *in vivo* (Lazaris-Karatzas *et al*., 1990; Ruggero and Pandolfi, 2003), arguing that translational control may be causally related to tumor formation. However eIF4E function, regardless of phosphorylation state, is also tonically inhibited by the 4E binding proteins (4E-BP1, 2 and 3) (Figure 1). In turn this inhibition is reversed by mTOR phosphorylation of the 4E-BPs, which is mediated by PI-3K via protein kinase B (PKB/Akt) (Figure 1). Thus eIF4E can act as a convergence point between Ras and Akt signaling in the control of translation (Miron *et al*., 2001). Such a model is supported by the data in glial cells demonstrating that the recruitment of oncogenic mRNAs to ribosomes occurs only with elevated combined Ras and Akt signaling, and not Akt signaling alone, indicating that in the context of elevated Akt signaling additional Ras activity enables the mRNA recruitment to occur (Rajasekhar *et al*., 2003). Whether other oncogenic signaling pathways can have a similar effect on the expression and activation of eIF4E, and therefore substitute for Ras activation in this regard is not known. An important goal of current investigation is determining how Ras and Akt activities impact on translational control and to unravel the epistatic role of essential components of the translation regulatory network in Ras and Akt cooperative oncogenesis.

Critical role of PI3-kinase signaling in the control of protein biosynthesis

The activation of PI-3K and the production of the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3) from PtdIns(4,5)P2 triggers the recruitment and activation of Akt by the phosphatidylinositol-dependent kinases, PDK1 and PDK2 (Brazil and Hemmings, 2001). PtdIns(3,4,5)P3 production is counteracted by the lipid phosphatase PTEN (*p*hosphatase and *ten*sin homologue deleted from chromosome 10) tumor suppressor (reviewed in Di Cristofano and Pandolfi, 2000; see following paragraphs; Figure 1).

mTOR

Akt activation is thought to result in the phosphorylation and inactivation of the tuberous sclerosis complex (TSC) tumor suppressor (see below), leading to the

*Correspondence: E Holland, E-mail: hollande@mskcc.org,
P Pandolfi, p-pandolfi@ski.mskcc.org,
N Sonenberg, nahum.sonenberg@mcgill.ca,
G Thomas, george.thomas@fmi.ch
Authors are listed in alphabetical order

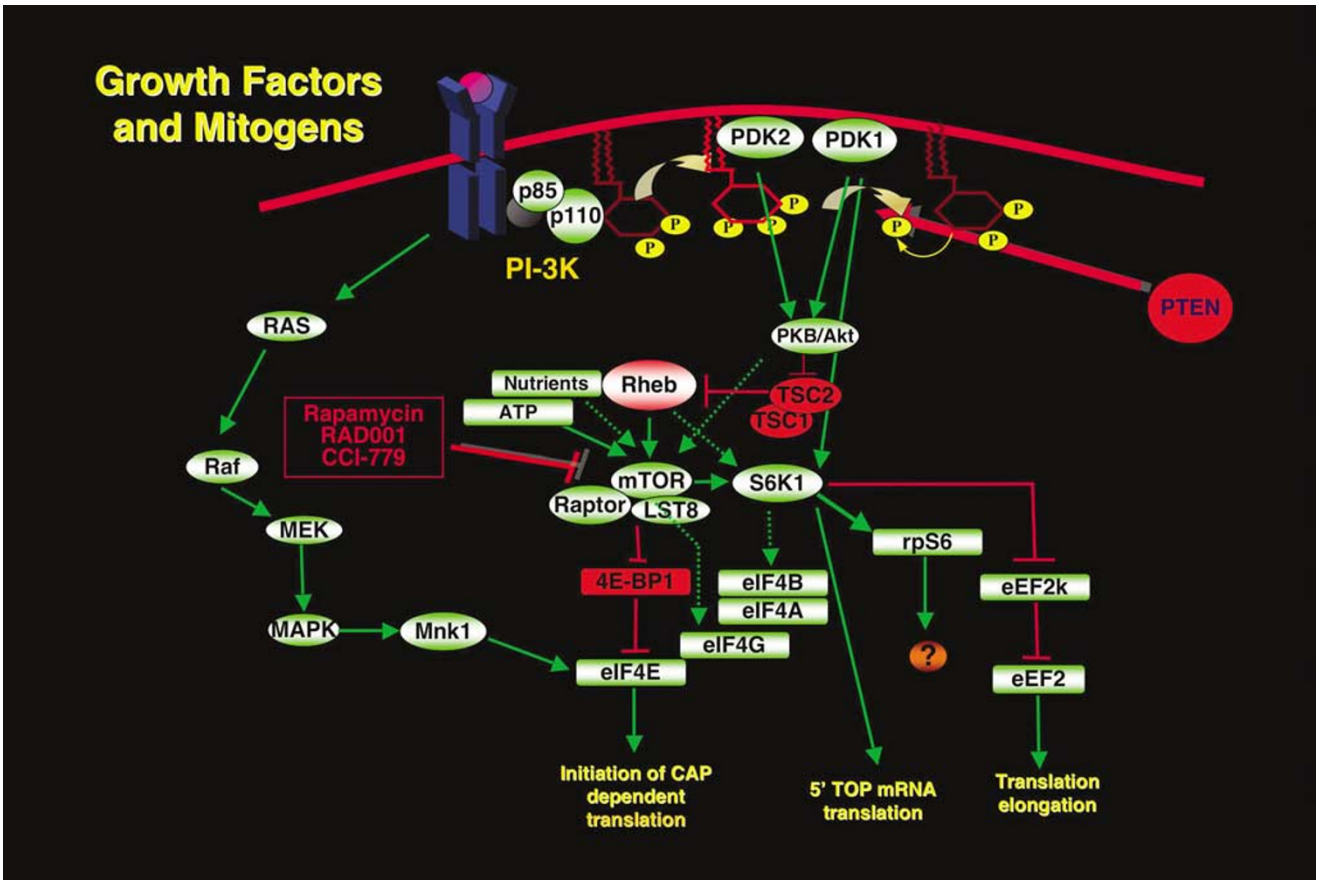

Figure 1 Ras- and PI3-kinase-dependent control of protein translation

activation of the mTOR signaling pathway (Figure 1). mTOR phosphorylates S6 ribosomal protein kinases (S6K1 and 2) and the 4E-BPs. S6K phosphorylation results in its activation, whereas phosphorylation of 4E-BP disrupts its interaction with eIF4E allowing eIF4E to bind and form a functional initiation complex (Figure 1). mTOR is emerging as a critical player in growth factor controlled translation, having been charged with integrating nutrient and energy homeostasis with the mitogenic input. Whether the effects of the TSC tumor suppressor are directly on mTOR or its downstream targets is still controversial. For example, insulin does not cause a change in mTOR kinase activity measured against S6K1 as an *in vitro* substrate (Dennis *et al*., 2001), suggesting that insulin might instead influence the composition and signaling capabilities of factors that are bound to mTOR, such as its recently described binding partners raptor and mLST8, rather than mTOR kinase activity itself (Hara *et al*., 2002; Kim *et al*., 2002; Loewith *et al*., 2002). In addition, it may be that the TSC tumor suppressor operates downstream of mTOR through an as yet undescribed mechanism (Jaeschke *et al*., 2002). Recent identification of the proto-oncogene Rheb (Ras homologue enriched in brain), an effector of TSC tumor suppressor function (Garami *et al*., 2003; Saucedo *et al*., 2003; Stocker *et al*., 2003; Zhang *et al*., 2003) should aid in delineating these interactions (Figure 1 and see below). Growth factors such as insulin also positively regulate nutrient uptake, including glucose and amino acids (Munoz *et al*., 1995; McDowell *et al*., 1998; Jefferson and Kimball, 2001). In particular, branched-chain amino acids, most notably leucine, exert a potent stimulatory effect on protein synthesis, an effect that is in part mediated by mTOR and its downstream effectors, the S6Ks and 4E-BPs (Kimball *et al*., 1999; Schmelzle and Hall, 2000; Gingras *et al*., 2001b) (Figure 1). In addition, it has been shown recently that mTOR activity is directly controlled by homeostatic levels of ATP (Dennis *et al*., 2001) as well as phosphatidic acid (Fang *et al*., 2001). Thus, mTOR appears to act as a permissive factor mediating protein synthesis according to the level of translational and energy precursors (Dennis *et al*., 1999, 2001). Consistent with this model, mTOR and PI-3K have been proposed to regulate autophagy negatively (Blommaart *et al*., 1995; Shigemitsu *et al*., 1999; Petiot *et al*., 2000), whereas PTEN has been suggested to have a positive effect (Arico *et al*., 2001).

TSC and Rheb

Recent reports have demonstrated constitutive activation of the mTOR/S6K1 pathway in cells lacking the TSC tumor suppressor function (Jaeschke *et al*., 2002;

Kenerson *et al*., 2002; Kwiatkowski *et al*., 2002). Human TSC is an autosomal dominant multiorgan disorder caused by germline mutations of the *TSC1* or *TSC2* genes (encoding hamartin and tuberin proteins, respectively) and characterized by mental retardation and hamartomas in various organs (Gomez *et al*., 1999). Interestingly, hamartomatous lesions have also been observed in patients with one of three related autosomal dominant disorders associated with germline *PTEN* mutations: Cowden disease, Lhermitte–Duclos disease and Bannayan–Zonana syndrome. These syndromes share specific developmental defects, hamartomatous lesions in the colonic mucosa as well as increased susceptibility to cancer (reviewed in Cantley and Neel, 1999; Di Cristofano and Pandolfi, 2000). TSC1 and TSC2 proteins form a heterodimeric functional complex that is inactive in the absence of either protein (Tee *et al*., 2002). The TSC tumor suppressor provides a further link between growth factor, nutrient signaling and the mTOR/S6K/4E-BP pathway (Gao and Pan, 2001; Inoki *et al*., 2002). In support of this notion, the overexpression of TSC inhibits nutrient- and growth factor-induced S6K1 activation and 4E-BP1 phosphorylation (Inoki *et al*., 2002; Jaeschke *et al*., 2002; Manning *et al*., 2002). In contrast, a variant of TSC2, harboring a point mutation in the GTPase-activating domain, a mutation associated with the human disease, has no inhibitory effect on S6K1 activation when overexpressed with TSC1 (Jaeschke *et al*., 2002). In addition, recent studies have shown that proto-oncogene Rheb is epistatically dominant to TSC1/2 and that TSC2 is the GTPase for Rheb (Garami *et al*., 2003; Saucedo *et al*., 2003; Stocker *et al*., 2003; Zhang *et al*., 2003).

Role of S6K activation and S6 phosphorylation in translation control and tumorigenesis

Accumulating evidence links the activation of PI3K-Akt to the two intracellular events that are regulated by mTOR: phosphorylation of S6K1 and 4E-BP1. However, growth factor stimulation can lead to S6K1 activation via mTOR as well as through mTOR-independent PI3K-PDK1 signaling inputs (Pullen and Thomas, 1997). In addition, neither Ca^{+2}, amino acids nor TPA treatment of cells affect Akt activation despite leading to acute S6K1 activation. Importantly, the activity of S6K1 is markedly upregulated in tumors that carry mutations in PTEN and TSC1/2 (reviewed in Ruggero and Pandolfi, 2003). Indeed, the overgrowth phenotype in the *Drosophila* eye caused by the loss of TSC function is totally suppressed in a dS6K-deficient background (Radimerski *et al*., 2002). More importantly, reducing dS6K signaling rescues early larval lethality associated with the loss of dTsc1/2 function (Radimerski *et al*., 2002), arguing that the S6K1 and 2 are promising targets for the treatment of TSC. A comparison of the S6K1 sequence with that of S6K2 reveals that they are highly homologous, sharing 82% identity in the catalytic, linker and autoinhibitory domains, as well as having all the same critical phosphorylation sites (Stocker *et al*., 2003). S6K1 activation proceeds first by the phosphorylation of the S/T-P sites in the autoinhibitory domain by mTOR, which acts to facilitate mTOR phosphorylation of T389, allowing PDK1 to dock on T389 and phosphorylate T229 (Biondi *et al*., 2001), resulting in S6K1 activation (Pullen *et al*., 1998).

The principal substrate of the S6Ks is the ribosomal protein S6, one of 30 distinct ribosomal proteins, which together with 18S rRNA, constitutes the smaller 40S ribosomal subunit (Wool *et al*., 1996). S6 has been localized to the small head region of the 40S subunit, near the mRNA/tRNA binding site, consistent with being one of the few 40S ribosomal proteins that has been crosslinked to 28S rRNA of the larger 60S ribosomal subunit (Volarevic and Thomas, 2001). The importance of S6 in ribosome function and protein translation is underscored by the conditional deletion of S6 in mice (Volarevic *et al*., 2000), which results in a block in ribosome biogenesis and cell cycle progression. The regulation of S6 phosphorylation is made more complex due to the presence of S6K2 (Shima *et al*., 1998).

S6 phosphorylation has been hypothesized to be involved in the translation of a specific class of mRNAs, TOP (*t*erminal *o*ligopyrimidine *t*ract in the 5′ untranslated region (5′UTR)) mRNA (Jefferies *et al*., 1994, 1997; Terada *et al*., 1994;). However recent studies from MEFs, derived from mice deficient for $S6K1^{-/-}/S6K2^{-/-}$, demonstrate that if these two kinases are implicated there must be parallel pathways involved as well (Pende, in press). The 5′TOP class of mRNAs includes most notably ribosomal proteins and elongation factors 1A and 2A (eEF1A1 and eEF1A2). 5′TOP mRNAs are regulated at the translation level as they are stored in mRNP particles (inactive translational particles), and shift to actively translating polysomes upon mitogenic stimulation (Meyuhas, 2000). The proteins encoded by 5′TOP genes have been shown to act themselves as proto-oncogenes. As an example, eEF1A2 has been found amplified in primary ovarian tumors and its overexpression is oncogenic in fibroblast cell lines and in xenograft tumor models (Anand *et al*., 2002). Therefore, deregulation in 5′TOP gene expression may have direct consequences on cancer initiation. Furthermore, given the fact that the majority of known 5′TOP genes all code for proteins that can upregulate protein synthesis, a positive feedback loop may exist by which an increase in S6 phosphorylation may result in an upregulation in total protein synthesis in the cell. S6 is phosphorylated when quiescent cells re-enter the cell cycle in response to mitogens (Thomas *et al*., 1979). Phosphorylation of S6 has been observed both *in vivo*, during liver regeneration, as well as in various cell lines (Gressner and Wool, 1974; Kozma and Thomas, 1994). As S6 phosphorylation levels increase in proportion to protein synthesis levels, it has been proposed that S6 could be an important regulator of cell growth. This has been in part substantiated in *Drosophila*, as inactivation of the dS6K results in a decrease of body size and a defect in cell growth (Montagne *et al*., 1999), whereas ectopic overexpression drives cell growth. Although there is no data to indicate that this phenotype is due to alterations

in translation of TOP genes, coexpression of S6 phosphorylation site mutants, which cannot be phosphorylated, block the dS6 kinase growth response in the wing (Radimerski and Thomas, unpublished observation). In addition, it has been speculated that S6 phosphorylation may also act to protect ribosomes from autophagic degradation (Blommaart *et al*., 1995), which is concomitantly downregulated as anabolic pathways become induced by growth factors (Seglen and Bohley, 1992; Blommaart *et al*., 1997).

Control of translation initiation in human cancer

In numerous cancers, general protein synthesis rates and the expression of several translation components are significantly elevated, supporting the potential importance of translational control in tumor progression (Hershey and Miyamoto, 2000). The increase in protein synthesis correlates with enhanced formation of the eIF4F initiation complex, which binds to the 5′ cap structure of eukaryotic mRNAs (Gingras *et al*., 1999b). The cap consists of the structure m^7GpppX (where X is any nucleotide). eIF4F is a trimeric complex comprised of eIF4G, eIF4A and eIF4E (Figure 1), which mediate the binding of the mRNA to the 43S preinitiation complex to form the 48S initiation complex (Pestova *et al*., 2001). eIF4E binds directly to the cap structure and is essential for the viability in yeast (Altmann *et al*., 1987). eIF4G is a scaffolding protein, which binds eIF4E and eIF4A and directly interacts with eIF3 of the 43S preinitiation complex (Gingras *et al*., 1999b). eIF4A serves as an ATP-dependent helicase. eIF4A functionally interacts with a cofactor, eIF4B, an initiation factor that promotes the binding of mRNA to 40S ribosomes *in vitro* and promotes ATP-dependent helicase activity of eIF4A (Rozen *et al*., 1990; Raught *et al*., 2004), to unwind secondary structures present within the 5′UTRs of certain mRNAs, until the initiator AUG. eIF4E appears to be the limiting factor in eIF4F complex formation (Duncan *et al*., 1987) and to be highly regulated upon growth factor stimulation (see below).

Enhanced protein synthesis in response to mitogens, hormones and growth factors is closely linked to increased eIF4F formation for the translation of a specific subset of mRNAs that contain highly structured (G + C-rich) 5′UTRs (Koromilas *et al*., 1992). Increased eIF4F formation is thought to contribute to the unwinding of these mRNAs, facilitating ribosome binding. Many structured mRNAs generally encode for growth-promoting genes including myc, ODC and FGF-2 (van der Velden and Thomas, 1999).

The activity of eIF4E as part of the eIF4F complex formation is regulated by growth factors through two distinct mechanisms. The first involves phosphorylation by Mnk1 (Waskiewicz *et al*., 1999), an event requiring binding of the kinase to the carboxyl-terminus of eIF4G (Pyronnet *et al*., 1999). Phosphorylation of eIF4E at serine 209 is important for eIF4E's activity and is thought to play a key role in its ability to promote tumorigenesis (Scheper and Proud, 2002). The biological importance of eIF4E phosphorylation has been demonstrated in *Drosophila* (Lachance *et al*., 2002), where mutation of eIF4E at the equivalent Ser209Ala, results in a delay in development and smaller flies (Lachance *et al*., 2002). The second involves a family of small repressor proteins termed the eIF4E binding proteins (4E-BPs) (Raught *et al*., 2000b). The 4E-BPs (4E-BP1, 4E-BP2, 4E-BP3) are small unstructured polypeptides that bind eIF4E, thereby preventing the formation of the eIF4F complex and its binding to the cap. Structural studies indicate that the 4E-BPs behave as molecular mimics of eIF4G by competing for binding to eIF4E (Marcotrigiano *et al*., 1999).

The regulation of eIF4E binding to the cap is an important target for potential therapeutic intervention for cancer (Meric and Hunt, 2002; see below), stemming from the finding that overexpression of eIF4E induces malignant transformation of rodent fibroblasts (Lazaris-Karatzas *et al*., 1990; Lazaris-Karatzas *et al*., 1992), as well as *in vivo* the transgenic mouse (Ruggero and Pandolfi, 2003) and that eIF4E expression is significantly increased in many cancers (De Benedetti and Harris, 1999). Importantly, the binding of eIF4E by the 4E-BPs partially reverts the eIF4E-induced phenotype (Rousseau *et al*., 1996), demonstrating that the 4E-BPs behave as tumor suppressors.

The PI3K-Akt-mTOR signaling pathway intimately links eIF4F complex formation and the 4E-BPs to cancer (Hidalgo and Rowinsky, 2000). As previously mentioned, mTOR directly phosphorylates the 4E-BPs and S6Ks. Phosphorylatable targets of mTOR also include eIF4G (Raught *et al*., 2000a) and eIF4B. eIF4B is also a direct target of S6K (Raught *et al*., 2004). Since the phosphorylation of S6 by S6K may not be the only mechanism by which the translation of TOP mRNAs is controlled, it may be that S6K1/2 mediate cell growth is also controlled through eIF4B phosphorylation. Furthermore, because inhibition of mTOR by rapamycin and its analogous (effective antitumoral agents, see following paragraphs) reduces the phosphorylation of several translational targets, including eIF4G and eIF4B, it adds further merit to the hypothesis that translational initiation plays a critical role in tumorigenesis (Hershey and Miyamoto, 2000).

The 4E-BPs are hyperphosphorylated in PTEN-mutated tumors (Wu *et al*., 1998) and phosphorylation is regulated by several extracellular stimuli, particularly growth factors, hormones and stress (Gingras *et al*., 1999a, 2001a). 4E-BP1, the best characterized of the BPs, undergoing phosphorylation in a stepwise manner at T37, T46, S65, T70, S83 and S112. Phosphorylation of S65 and T70 are markedly increased by serum stimulation and inhibited by rapamycin (Gingras *et al*., 2001a). Phosphorylation of T70 precedes that of S65 and both events require prior phosphorylation of T37 and T46 (Gingras *et al*., 2001a). The biological importance of 4E-BP1 phosphorylation is emphasized by the observation that nonphosphorylatable 4E-BP1 mutants prevent colony formation and activate apoptosis in rodent fibroblasts (Li *et al*., 2002).

Role of PTEN in tumor suppression and translation control

The *PTEN* gene is located on chromosome 10q23, a genomic region frequently lost in human cancers. Somatic deletions or mutations of this gene have been identified in a large fraction of tumors, frequently in glioblastomas and prostate cancers, placing *PTEN* among the most commonly mutated genes in human cancer (Cantley and Neel, 1999; Di Cristofano and Pandolfi, 2000). Moreover, it has been reported that primary tumors often show loss or alteration of at least one *PTEN* allele (e.g. 70% of primary prostate cancers; Gray *et al*., 1998; Whang *et al*., 1998) supporting *PTEN* involvement in tumor progression, while homozygous inactivation of *PTEN* is generally associated with advanced cancer and metastasis (Cantley and Neel, 1999; Di Cristofano and Pandolfi, 2000). However, as dictated by Knudson's 'two-hit' hypothesis, the analysis of cancer samples for mutations in *PTEN* has been initially performed searching for biallelic inactivation of the gene, which pointed at complete *PTEN* inactivation as a late event in cancer progression. Thus, the consequence of loss/mutation in one *PTEN* allele in carcinomas *in situ* or primary cancers may have been underestimated. It could be postulated that while the loss of one *PTEN* allele may be playing a key role in tumor initiation, complete loss of *PTEN* may favor metastatization in advanced cancers. Characterization of tumorigenesis and cooperative oncogenesis in $Pten^{+/-}$ and *Pten* hypomorph ($Pten^{hy}$) mutant mice by the Pandolfi lab supports this notion and demonstrate that Pten is haploinsufficient in some of the biological functions (Di Cristofano *et al*., 1998, 1999, 2001; Trotman *et al*., 2003).

The discovery that PIP-3 is the main *in vivo* substrate of PTEN placed this phosphatase into a well-defined pathway. PIP-3 levels are very low in quiescent cells, but rapidly increase upon stimulation by growth factors, through PI3-K activation. The role of PTEN is to keep the levels of PIP-3 low by dephosphorylating it at the D3 position. The loss of PTEN function results in increased PIP-3 levels and subsequent Akt hyperactivation (Stambolic *et al*., 1998; Di Cristofano *et al*., 1999, 2001; Backman *et al*., 2001; Kwon *et al*., 2001; Suzuki *et al*., 2001; Crackower *et al*., 2002; Li *et al*., 2002). This leads to elevated mTOR activity and enhanced phosphorylation of several translational targets (4E-BPs, eIF4G, eIF4B, S6K1). As a consequence, the biological outcomes of PTEN mutations include alterations in cell growth and cell size (Backman *et al*., 2002).

The importance of mTOR activation in tumorigenesis driven by *PTEN* functional loss is underscored by studies with CCI-779 (a rapamycin analogue; see below). PTEN-deficient cancer cell lines are sensitive to pharmacological inhibition of mTOR by CCI-779, and enhanced tumor growth caused by constitutive activation of Akt/mTOR is reversed by CCI-779 treatment (Neshat *et al*., 2001). In addition, rapamycin can reduce endometrial atypical hyperplasia in *Pten* heterozygous mice (Podsypanina *et al*., 2001), substantiating the notion that mTOR acts downstream the PTEN-Akt pathway in tumorigenesis.

Finally and crucial to the argument for the importance of translational mechanisms in cancer, protein components of oncogenic pathways are encoded by mRNAs that are regulated at the translational level. Numerous published studies have shown that individual oncogenic mRNAs are translationally regulated (reviewed and tabulated in this issue by Rajasekhar and Holland, Table 2). However, in order for such an effect to have oncogenic potential, it must be specific to oncogenic mRNAs and not affect the entire spectrum of mRNAs. The global analysis of mRNAs regulated at the translational level as a function of combined Ras and Akt signaling in glia provides some insight into the specificity and magnitude of this phenomenon. In this study, approximately 5% of mRNAs were differentially recruited to polysomes (threefold or greater: in some cases more than 20-fold). Furthermore, the proteins encoded by these mRNAs are highly enriched in cancer-promoting functions (Rajasekhar *et al*., 2003).

Therapeutic implications

The immunosuppressant drug rapamycin, which forms a complex with the immunophilin prolyl isomerase FKBP12, specifically inhibits mTOR kinase activity (Huang and Houghton, 2002). Rapamycin inhibits the proliferation of several types of mammalian cells, most dramatically B- and T-lymphocytes, and results in G1 arrest (Wicker *et al*., 1990; Terada *et al*., 1993). Owing to its inhibitory effects on lymphocyte proliferation, rapamycin is a potent immunosuppressant and effectively prevents allograft rejection. However, rapamycin has also been extensively characterized as a potential anticancer drug, displaying potent cytostatic effects in several cancer cell lines of various histological origins and, less frequently, marked proapoptotic activity (Huang and Houghton, 2002). In particular, neuroblastoma and glioblastoma cells are sensitive to rapamycin (Hosoi *et al*., 1998). Rapamycin also prevents mitogen-stimulated downregulation of $p27^{Kip1}$ (Nourse *et al*., 1994; Luo *et al*., 1996), a function that may be of critical relevance in the treatment of prostate cancer (CaP) in view of the role of $p27^{Kip1}$ functional loss in CaP in combination with Pten loss (see next paragraph). Indeed, CCI-779 (see below) reverses doxorubicin resistance conferred by PTEN status in prostate cancer cells (Grunwald *et al*., 2002), and rapamycin reduces the proliferation rate of human pancreatic cancer cells, which display a constitutively active mTOR-S6K1/4E-BP1 signaling pathway (Grewe *et al*., 1999). Owing to some of the side effects of rapamycin, analogues such as CCI-779 (an ester of rapamycin) or RAD001 (an oral available analogue of rapamycin also known as Everolimus) have been developed (Huang and Houghton, 2002). CCI-779 is currently in Phase III clinical trials in cancer patients in both United States and Europe, and

RAD001 is currently in Phase I trials for the treatment of TSC syndromes and solid tumors that display *PTEN* mutations or deficiencies. Indeed, since rapamycin and its analogues affect indirectly cap-dependent translation by inhibiting the phosphorylation of 4E-BPs, eIF4G and eIF4B, it has also been suggested that these proteins may provide even more specific targets for potential chemotherapeutic intervention (Meric and Hunt, 2002). Thus, the *in vivo* genetic assessment of the relevance in cancer pathogenesis of each event/component in the translation control network is of paramount importance for the identification of novel druggable targets.

References

Altmann M, Handschin C and Trachsel H. (1987). *Mol. Cell. Biol.*, **7,** 998–1003.

Anand N, Murthy S, Amann G, Wernick M, Porter LA, Cukier IH, Collins C, Gray JW, Diebold J, Demetrick DJ and Lee JM. (2002). *Nat. Genet.*, **31,** 301–305.

Antonetti DA, Kimbal SR, Horetsky RL and Jefferson LS. (1993). *J. Biol. Chem.*, **268,** 25277–25284.

Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P and Ogier-Denis E. (2001). *J. Biol. Chem.*, **276,** 35243–35246.

Backman S, Stambolic V and Mak T. (2002). *Curr. Opin. Neurobiol.*, **12,** 516–522.

Backman S, Stambolic V, Suzuki A, Haight J, Elia A, Pretorius J, Tsao MS, Shannon P, Bolon B, Ivy GO and Mak TW. (2001). *Nat. Genet.*, **4,** 396–403.

Biondi RM, Kieloch A, Currie RA, Deak M and Alessi DR. (2001). *EMBO J.*, **20,** 4380–4390.

Blommaart EF, Luiken JJ and Meijer AJ. (1997). *Histochem. J.*, **29,** 365–385.

Blommaart EF, Luiken JJ, Blommaart PJ, van Woerkom GM and Meijer AJ. (1995). *J. Biol. Chem.*, **270,** 2320–2326.

Brazil DP and Hemmings BA. (2001). *Trends Biochem. Sci.*, **26,** 657–664.

Cantley LC and Neel BG. (1999). *Proc. Natl. Acad. Sci. USA*, **96,** 4240–4245 Review.

Crackower MA, Oudit GY, Kozieradzki I and Sarao R. (2002). *Cell*, **110,** 737–749.

De Benedetti A and Harris AL. (1999). *Int. J. Biochem. Cell Biol.*, **31,** 59–72.

Dennis PB, Fumagalli S and Thomas G. (1999). *Curr. Opin. Genet. Dev.*, **9,** 49–54.

Dennis PB, Jaeschke A, Saitoh M, Fowler B, Kozma SC and Thomas G. (2001). *Science*, **294,** 1102–1105.

Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C and Pandolfi PP. (2001). *Nat. Genet.*, **27,** 222–224.

Di Cristofano A, Kotsi P, Peng YF, Cordon-Cardo C, Elkon K and Pandolfi PP. (1999). *Science*, **285,** 2122–2125.

Di Cristofano A, Pesce B, Cordon-Cardo C and Pandolfi PP. (1998). *Nat. Genet.*, **19,** 348–355.

Di Cristofano A and Pandolfi PP. (2000). *Cell*, **100,** 387–390.

Duncan R, Milburn SC and Hershey JW. (1987). *J. Biol. Chem.*, **262,** 380–388.

Fang Y, Vilella-Bach M, Bachmann R, Flanigan A and Chen J. (2001). *Science*, **294,** 1942–1945.

Gao X and Pan D. (2001). *Genes Dev.*, **15,** 1383–1392.

Garami A, Zwartkruis FJ, Nobukuni T, Joaquin M, Roccio M, Stocker H, Kozma SC, Hafen E, Bos JL and Thomas G. (2003). *Mol. Cell*, **11,** 1457–1466.

Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R and Sonenberg N. (1999a). *Genes Dev.*, **13,** 1422–1437.

Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, Burley SK, Polakiewicz RD, Wyslouch-Cieszynska A, Aebersold R and Sonenberg N. (2001a). *Genes Dev.*, **15,** 2852–2864.

Gingras AC, Raught B and Sonenberg N. (1999b). *Annu. Rev. Biochem.*, **68,** 913–963.

Gingras AC, Raught B and Sonenberg N. (2001b). *Genes Dev.*, **15,** 807–826.

Gomez M, Sampson J and Whittemore l. (1999). *Tuberous Sclerosis*, Oxford University Press: Oxford, Vol. 340.

Gray IC, Stewart LM, Phillips SM, Hamilton JA, Gray NE, Watson GJ, Spurr NK and Snary D. (1998). *Br. J. Cancer*, **78,** 1296–1300.

Gressner AM and Wool IG. (1974). *J. Biol. Chem.*, **249,** 6917–6925.

Grewe M, Gansauge F, Schmid RM, Adler G and Seufferlein T. (1999). *Cancer Res.*, **59,** 3581–3587.

Grunwald V, DeGraffenried L, Russel D, Friedrichs WE, Ray RB and Hidalgo M. (2002). *Cancer Res.*, **62,** 6141–6145.

Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J and Yonezawa K. (2002). *Cell*, **110,** 177–189.

Hershey JWB and Miyamoto S. (2000). *Translational Control of Gene Expression*, Sonenberg N, Hershey JWB, Mathews MB (eds). Cold Spring Harbor Laboratory Press: New York, pp. 637–654.

Hidalgo M and Rowinsky EK. (2000). *Oncogene.*, **19,** 6680–6686.

Hosoi H, Dilling MB, Liu LN, Danks MK, Shikata T, Sekulic A, Abraham RT, Lawrence JC and Houghton PJ. (1998). *Mol. Pharmacol.*, **54,** 815–824.

Huang S and Houghton PJ. (2002). *Curr. Opin. Invest. Drugs*, **3,** 295–304.

Inoki K, Li Y, Zhu T, Wu J and Guan KL. (2002). *Nat. Cell Biol.*, **4,** 648–657.

Jaeschke A, Hartkamp J, Saitoh M, Roworth W, Nobukuni T, Hodges A, Sampson J, Thomas G and Lamb R. (2002). *J. Cell Biol.*, **159,** 217–224.

Jefferies HB, Fumagalli S, Dennis PB, Reinhard C, Pearson RB and Thomas G. (1997). *EMBO J.*, **16,** 3693–3704.

Jefferies HB, Reinhard JC, Kozma SC and Thomas G. (1994). *Proc. Natl. Acad. Sci. USA*, **91,** 4441–4445.

Jefferson LS and Kimball SR. (2001). *J. Nutr.*, **131,** 2460S–2466S (discussion 2486S–2467S).

Kenerson HL, Aicher LD, True LD and Yeung RS. (2002). *Cancer Res.*, **62,** 5645–5650.

Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P and Sabatini DM. (2002). *Cell*, **110,** 163–175.

Kimball SR, Shantz LM, Horetsky RL and Jefferson LS. (1999). *J. Biol. Chem.*, **274,** 11647–11652.

Koromilas AE, Lazaris-Karatzas A and Sonenberg N. (1992). *EMBO J.*, **11,** 4153–4158.

Kozma SC and Thomas G. (1994). *Cancer Biol.*, **5,** 255–260.

Kwiatkowski DJ, Zhang H, Bandura JL, Heiberger KM, Glogauer M, el-Hashemite N and Onda H. (2002). *Hum. Mol. Genet.*, **11,** 525–534.

Kwon CH, Zhu X, Zhang J, Knoop LL, Tharp R, Smeyne RJ, Eberhart CG, Burger PC and Baker SJ. (2001). *Nat. Genet.*, **4,** 404–411.

Lachance PE, Miron M, Raught B, Sonenberg N and Lasko P. (2002). *Mol. Cell. Biol.*, **22,** 1656–1663.
Lazaris-Karatzas A, Montine KS and Sonenberg N. (1990). *Nature*, **345,** 544–547.
Lazaris-Karatzas A, Smith MR, Frederickson R.M, Jaramillo ML, Liu YL, Kung HF and Sonenberg N. (1992). *Genes Dev.*, **6,** 1631–1642.
Li S, Sonenberg N, Gingras AC, Peterson M, Avdulov S, Polunovsky VA and Bitterman PB. (2002). *Mol. Cell. Biol.*, **22,** 2853–2861.
Loewith R, Jacinto E, Wullschleger S, Lorberg A, Crespo JL, Bonenfant D, Oppliger W, Jenoe P and Hall MN. (2002). *Mol. Cell*, **10,** 457–468.
Luo Y, Marx SO, Kiyokawa H, Koff A, Massague J and Marks AR. (1996). *Mol. Cell. Biol.*, **16,** 6744–6751.
Manning BD, Tee AR, Logsdon MN, Blenis J and Cantley LC. (2002). *Mol. Cell*, **10,** 151–162.
Marcotrigiano J, Gingras AC, Sonenberg N and Burley SK. (1999). *Mol. Cell*, **3,** 707–716.
McDowell HE, Eyers PA and Hundal HS. (1998). *FEBS Lett.*, **441,** 15–19.
Meric F and Hunt KK. (2002). *Mol. Cancer Ther.*, **1,** 971–979.
Meyuhas O. (2000). *Eur. J. Biochem.*, **267,** 6321–6330.
Miron M, Verdu J, Lachance PE, Birnbaum MJ, Lasko PF and Sonenberg N. (2001). *Nat. Cell Biol.*, **3,** 596–601.
Montagne J, Stewart MJ, Stocker H, Hafen E, Kozma SC and Thomas G. (1999). *Science*, **285,** 2126–2129.
Munoz M, Sweiry JH and Mann GE. (1995). *Exp. Physiol.*, **80,** 745–753.
Neshat MS, Mellinghoff IK, Tran C, Stiles B, Thomas G, Petersen R, Frost P, Gibbons JJ, Wu H and Sawyers CL. (2001). *Proc. Natl. Acad. Sci. USA*, **98,** 10314–10319.
Nourse J, Firpo E, Flanagan WM, Coats S, Polyak K, Lee MH, Massague J, Crabtree GR and Roberts JM.. (1994). *Nature*, **372,** 570–573.
Pestova TV, Kolupaeva VG, Lomakin IB, Pilipenko EV, Shatsky IN, Agol VI and Hellen CU. (2001). *Proc. Natl. Acad. Sci. USA*, **98,** 7029–7036.
Petiot A, Ogier-Denis E, Blommaart EF, Meijer AJ and Codogno P. (2000). *J. Biol. Chem.*, **275,** 992–998.
Podsypanina K, Lee RT, Politis C and Hennessy I. (2001). *Proc. Natl. Acad. Sci. USA*, **98,** 10320–10325.
Pullen N, Dennis PB, Andjelkovic M, Dufner A, Kozma S, Hemmings BA and Thomas G. (1998). *Science*, **279,** 707–710.
Pullen N and Thomas G. (1997). *Fed. Eur. Biochem. Soc. Lett.*, **410,** 78–82.
Pyronnet S, Imataka H, Gingras AC, Fukunaga R, Hunter T and Sonenberg N. (1999). *EMBO J.*, **18,** 270–279.
Radimerski T, Montagne J, Rintelen F, Stocker H, van Der Kaay J, Downes CP, Hafen E and Thomas G. (2002). *Nat. Cell Biol.*, **4,** 251–255.
Rajasekhar VK, Viale A, Socci ND, Wiedmann M, Hu X and Holland EC. (2003). *Mol. Cell.*, **12,** 889–901.
Raught B, Gingras AC, Gygi SP, Imataka H, Morino S, Gradi A, Aebersold R and Sonenberg N. (2000a). *EMBO. J.*, **19,** 434–444.
Raught B, Gingras AC and Sonenberg N. (2000b). *Translational Control of Gene Expression*, Sonenberg N, Hershey JWB, Mathews MB (eds). Cold Spring Harbor Laboratory Press: New York, pp. 245–293.
Raught B, Peiretti F, Gingras AC, Livingstone M, Shahbazian D, Mayeur GL, Polakiewicz RD, Sonenberg N and Hershey JWB. (2004). *EMBO. J.*, in press.
Rousseau D, Gingras AC, Pause A and Sonenberg N. (1996). *Oncogene*, **13,** 2415–2420.
Rozen F, Edery I, Meerovitch K, Dever TE, Merrick WC and Sonenberg N. (1990). *Mol. Cell. Biol.*, **10,** 1134–1144.
Ruggero D and Pandolfi PP. (2003). *Nat. Rev. Cancer*, **3,** 175–192.
Saucedo LJ, Gao X, Chiarelli DA, Li L, Pan D and Edgar BA. (2003). *Nat. Cell Biol.*, **5,** 566–571 (Erratum in: Nat. Cell Biol. **5** , 680).
Scheper GC and Proud CG. (2002). *Eur. J. Biochem.*, **269,** 5350–5359.
Scheper GC, Morrice NA, Kleijn M and Proud CG. (2001). *Mol. Cell. Biol.*, **21,** 743–754.
Schmelzle T and Hall MN. (2000). *Cell*, **103,** 193–200.
Seglen PO and Bohley P. (1992). *Experientia*, **48,** 158–172.
Shigemitsu K, Tsujishita Y, Hara K, Nanahoshi M, Avruch J and Yonezawa K. (1999). *J. Biol. Chem.*, **274,** 1058–1065.
Shima H, Pende M, Chen Y, Fumagalli S, Thomas G and Kozma SC. (1998). *EMBO J.*, **17,** 6649–6659.
Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger JM, Siderovski DP and Mak TW. (1998). *Cell*, **95,** 29–39.
Stocker H, Radimerski T, Schindelholz B, Wittwer F, Belawat P, Daram P, Breuer S, Thomas G and Hafen E. (2003). *Nat. Cell Biol.*, **5,** 559–565.
Suzuki A, Yamaguchi MT, Ohteki T and Sasaki T. (2001). *Immunity*, **14,** 523–534.
Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC and Blenis J. (2002). *Proc. Natl. Acad. Sci. USA*, **99,** 13571–13576.
Terada N, Lucas JJ, Szepesi A, Franklin RA, Domenico J and Gelfand EW. (1993). *J. Cell Physiol.*, **154,** 7–15.
Terada N, Patel HR, Takase K, Kohno K, Nairn AC and Gelfand EW. (1994). *Proc. Natl. Acad. Sci. USA*, **91,** 11477–11481.
Thomas G, Siegmann M and Gordon J. (1979). *Proc. Natl. Acad. Sci. USA*, **76,** 3952–3956.
Trotman L, Niki M, Dotan Z, Koutcher J, Di Cristofano A, Xiao A, Khoo A, Brunet A, Roy-Burman P, Greenberg N, Van Dyke T, Cordon-Cardo C and Pandolfi PP. (2003). *Public Libr. Sci., Biol.*, **1,** 385–396.
van der Velden AW and Thomas AA. (1999). *Int. J. Biochem. Cell Biol.*, **31,** 87–106.
Volarevic S and Thomas G. (2001). *Prog. Nucleic Acid Res. Mol. Biol.*, **65,** 101–127.
Volarevic S, Stewart MJ, Ledermann B, Zilberman F, Terracciano L, Montini E, Grompe M, Kozma SC and Thomas G. (2000). *Science*, **288,** 2045–2047.
Waskiewicz AJ, Johnson JC, Penn B, Mahalingam M, Kimball SR and Cooper JA. (1999). *Mol. Cell. Biol.*, **19,** 1871–1880.
Whang HE, Wu X, Suzuki H, Reiter RE, Tran C, Vessella RL, Said JW, Isaacs WB and Sawyers CL. (1998). *Proc. Natl. Acad. Sci. USA*, **95,** 5246–5250.
Wicker LS, Boltz Jr RC, Matt V, Nichols EA, Peterson LB and Sigal NH. (1990). *Eur. J. Immunol.*, **20,** 2277–2283.
Wool IG, Chan YL and Glück A. (1996). *Translational Control*, Hershey JWB, Mathews MB, Sonenberg N (eds). Cold Spring Harbor Laboratory Press: New York, pp. 685–732.
Wu X, Senechal K, Neshat MS, Whang YE and Sawyers CL. (1998). *Proc. Natl. Acad. Sci. USA*, **95,** 15587–15591.
Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA and Pan D. (2003). *Nat. Cell Biol.*, **5,** 578–581.