Cell Cycle Protein Expression in Vascular Smooth Muscle Cells In Vitro and In Vivo Is Regulated Through Phosphatidylinositol 3-Kinase and Mammalian Target of Rapamycin

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Abstract—Cell cycle progression represents a key event in vascular proliferative diseases, one that depends on an increased rate of protein synthesis. An increase in phosphatidylinositol 3-kinase (PI 3-kinase) activity is associated with vascular smooth muscle cell proliferation, and rapamycin, which blocks the activity of the mammalian target of rapamycin, inhibits this proliferation in vitro and in vivo. We hypothesized that these 2 molecules converge on a critical pathway of translational regulation that is essential for successful upregulation of cell cycle–regulatory proteins in activated smooth muscle cells. p70S6 kinase, a target of PI 3-kinase and the mammalian target of rapamycin, was rapidly activated on growth factor stimulation of quiescent coronary artery smooth muscle cells and after balloon injury of rat carotid arteries. The translational repressor protein 4E-binding protein 1 was similarly hyperphosphorylated under these conditions. These events were associated with increases in the protein levels of cyclin B1, cyclin D1, cyclin E, cyclin-dependent kinase 1, cyclin-dependent kinase 2, proliferating cell nuclear antigen, and p21Cip1 in vivo and in vitro, whereas inhibition of the PI 3-kinase signaling pathway with either rapamycin or wortmannin blocked the upregulation of these cell cycle proteins, but not mRNA, and arrested the cells in vitro before S phase. In contrast to findings in other cell types, growth factor– or balloon injury–induced downregulation of the cell cycle inhibitor p27Kip1 was not affected by rapamycin treatment. These data suggest that cell cycle progression in vascular cells in vitro and in vivo depends on the integrity of the PI 3-kinase signaling pathway in allowing posttranscriptional accumulation of cell cycle proteins. (Arterioscler Thromb Vasc Biol. 2001;21:1152-1158.)

Key Words: cell cycle protein ■ S6 kinase ■ phosphatidylinositol 3-kinase ■ balloon injury ■ vascular smooth muscle

The entry of vascular cells into the cell cycle plays an important role in the pathogenesis of proliferative vascular diseases, such as postangioplasty restenosis, transplant vasculopathy, vein graft disease, and primary atherosclerosis.1-3 Cellular proliferation involves changes not only in the level of gene transcription but also in the rate of protein translation.4 We hypothesized that in addition to the generalized increases in protein synthesis necessary for cell growth, there is a highly regulated increase in the translation of certain mRNA species, including those that encode cell cycle proteins, and we also hypothesized that the molecular machinery regulating this translation represents a potential target for therapeutic intervention.

The increased protein translation observed during cell cycle progression is associated with phosphorylation of the ribosomal protein S6 and activation of translation factors, in particular, eukaryotic initiation factor 4E (eIF-4E).6 Under resting conditions, inactive eIF-4E is tightly bound to its repressor 4E-binding protein 1 (4E-BP1). Mitogens cause phosphorylation of 4E-BP1 and its dissociation from eIF-4E. The pathway consisting of the mammalian target of rapamycin (mTOR) and of the p70/p85-kDa S6 kinases (p70S6 kinase) appears to be crucial for the activation of these translation factors.7 mTOR, which can be blocked by the immunosuppressant rapamycin after the latter has formed a complex with the immunophilin FK-binding protein (FKBP),8 possesses kinase activity that is required for p70S6 kinase activation9 and may also be directly involved in 4E-BP1 phosphorylation.10 Phosphatidylinositol 3-kinase (PI 3-kinase) is another regulator involved in the activation of mTOR/p70S6 kinase.11 It has been suggested that PI 3-kinase and mTOR act via independent and parallel pathways.12
mTOR may sense nutrient availability within the cell and provide basal phosphorylation of p70S6 kinase, whereas PI 3-kinase may mediate subsequent mitogen-induced phosphorylation of p70S6 kinase. It has also been proposed that PI 3-kinase can act upstream from mTOR, thereby leading to activation of mTOR and p70S6 kinase.

In the present study, we tested the hypothesis that phosphorylation of 4E-BP1 and p70S6 kinase by PI 3-kinase and mTOR are critical events during the mitogenic stimulation of coronary artery smooth muscle cells (CASMCS) in vitro and in response to vascular injury in vivo. We further hypothesized that the translation of cell cycle genes, such as those encoding cyclin B1, cyclin D1, cyclin E, cyclin-dependent kinase (Cdk)1, Cdk2, proliferating cell nuclear antigen (PCNA), and p21<sup>Cip1</sup>, is dependent on the activation of these translational regulators and would be inhibited by their blockade. Because rapamycin activity has been linked to a stabilization of the Cdk inhibitor p27<sup>Kip1</sup> in other cell types, we also measured the effect of rapamycin treatment on p27<sup>Kip1</sup> protein levels in vitro and in vivo.

**Methods**

**Materials**

Antibodies used are as follows: rabbit polyclonal anti–cyclin B1 (M-20), anti–cyclin E (M-20), anti–Cdk2 (M2), anti–Cdk4 (C-22), anti-p27<sup>Kip1</sup> (N20), anti-p21<sup>Cip1</sup> (19), and anti-p70 S6 kinase (C-18); mouse monoclonal anti-cyclin D1 (HD11), anti-Cdk1 (17), anti-PCNA (PC10), and anti–PI 3-kinase (B-9); and goat polyclonal anti–4E-BP1 (N-19) (Santa Cruz Biotechnology). Antibodies were characterized via preincubation with specific blocking peptide, which did not reveal nonspecific cross-reaction in the blots.

**Cell Culture**

CASMCS, passages 4 to 8, were maintained in 0.5 ng/mL human epidermal growth factor, 5 μg/mL insulin, 2 ng/mL human fibroblast growth factor, 5% FBS, 50 μg/mL gentamicin, and 50 ng/mL amphotericin B according to supplier’s recommendations. Quiescence was achieved after confluence for 2 days by growth factor withdrawal for 24 hours. Murine BCH1 cells were also grown according to the supplier’s instructions (American Type Culture Collection).

**Metabolic Labeling Studies**

Tritiated thymidine and leucine labeling were used as previously described for measurements of DNA and protein synthesis, respectively.

**Flow Cytometry**

Cells were harvested by trypsinization, fixed overnight with 75% methanol, washed, and incubated with 100 μg/mL RNase (Oncogene) and 10 μg/mL propidium iodide in PBS for 1 hour at 37°C. Samples were analyzed for DNA content by using standard methods on a Coulter Epics XL-MCL flow cytometer. Data were computer-analyzed with Multiple Option Cell Cycle Fitting (version 2.50, Phoenix Flow Systems).

**Preparation of Cellular Lysates and Western Immunoblot Analysis**

Specific protein content in cell lysates was analyzed by Western blot as previously described. Briefly, supernatant was run on polyacrylamide gels and then blotted onto nitrocellulose (Hybon-EC, Amersham) by wet electrophoretic transfer. After blocking, blots were incubated with primary antibody (dilution 1:1000 for PI 3-kinase; 1:200 for PCNA, Cdk1, Cdk2, Cdk4, cyclin B1, cyclin D1, cyclin E, p27<sup>Kip1</sup>, p21<sup>Cip1</sup>, and p70S6 kinase; and 1:50 for 4E-BP1) for 1 hour at room temperature. Specific proteins were then detected by enhanced chemiluminescence (ECL, Amersham) after labeling with horseradish peroxidase–labeled secondary antibody according to the manufacturer’s instructions.

**Immune Complex Kinase Assay for p70S6 Kinase**

Cell lysates (250 μg protein) were labeled with anti-p70S6 kinase antibody. Protein G Plus/Protein A-Agarose (Calbiochem) was then added (30 μL of a 50% suspension), and incubation was continued for 1 hour at 4°C. Immunoprecipitates were washed, and phosphorylation of a substrate (AKRRLLSSLRA), modeled after the phosphorylation sites in S6 kinase, with [γ-<sup>32</sup>P]ATP (3000 Ci/mmol, DuPont NEN) was measured according to the manufacturer’s instructions (Upstate).

**Histone H1 Kinase Assay**

CASMCS lysates were labeled with anti-Cdk antibody, and immune complexes bound to protein A/G-agarose beads (Oncogene Sciences) were assayed by addition of kinase buffer, histone H1, and [γ-<sup>32</sup>P]ATP (3000 Ci/mmol, DuPont, NEN) as described previously. The samples were boiled for 5 minutes, electrophoresed through a 12% SDS-polyacrylamide gel, dried, and exposed to x-ray film.

**Analysis of mRNA Expression by RPA**

Total RNA was extracted by use of the RNeasy mini kit (Qiagen) and was quantified by absorbance at 260 nm. Cell cycle protein mRNAs were detected by using a RiboQuant MultiProbe RNase protection assay (RPA) system (Pharmingen). Multiprobes (hCC-1 and hCYC-1), containing the templates for cell cycle proteins and the housekeeping genes L-32 and GAPDH, were labeled with [γ-<sup>32</sup>P]UTP by using T7 RNA polymerase. Labeled probe (3×10<sup>5</sup> cpm) was hybridized to 2 μg of total RNA overnight at 56°C. The mRNA probe hybrids were treated with RNase and purified according to the RiboQuant protocol. Protected hybrids were resolved on a 6% polyacrylamide-Tris-borate-EDTA-urea gel by using the Seqi-Gen GT Nucleic Acid Electrophoresis Cell (Bio-Rad). Gels were dried and exposed to radiographic films overnight. Normalized densitometric results are expressed as percentage of the housekeeping genes (mean of GAPDH and LP-32) for each sample studied.

**In Vivo Balloon Injury of Rat Carotid Artery and In Vivo Treatment Protocol**

A 2F Fogarty catheter was used to produce vascular injury in male Sprague-Dawley rats (400 g) as previously described. At indicated times, the rats were euthanized, and both carotid arteries were perfused with saline, gently denuded of surrounding tissue, snap-frozen, and stored at −70°C until use. Rapamycin-treated animals were compared with control animals. For the treated animals, rapamycin (6 mg/kg body wt, Wyeth-Ayerst Research) suspended in 0.2% carboxymethylcellulose was injected once a day intraperitoneally, starting 4 days before injury to 1 day before the animals were euthanized. Control animals were treated with the same volume of vehicle.

**Statistical Analysis**

Data are given as mean±SEM. Statistical analysis was performed by ANOVA as appropriate. Post-test multiple comparison was performed by the method of Bonferroni. Statistical significance was accepted at the appropriate P level. Immunoblots, shown, were independently repeated at least 3 times.

**Results**

**Cell Cycle Protein Expression and Proliferation of CASMCs In Vitro Is Mediated Through PI 3-Kinase Signal Transduction**

We measured p70<sup>S6</sup> kinase activity in quiescent CASMCs and during stimulation of these cells with 0.5 ng/mL human epidermal growth factor, 5 μg/mL insulin, 2 ng/mL human fibroblast growth factor, and 5% FBS. The kinase activity of the enzyme rose within 10 minutes, reaching a stable plateau after 30 minutes for 8 hours, with a late fall after 24 hours of...
growth factor stimulation (data not shown). Pretreatment with either rapamycin (10 nmol/L) or wortmannin (10 nmol/L), inhibitors of mTOR and PI 3-kinase, respectively, successfully blocked p70 S6 kinase activation in quiescent CASMCs (14.3 ± 1.6 [quiescent] versus 4.1 ± 0.6 [rapamycin] and 5.8 ± 0.4 [wortmannin] pmol/min per milligram protein, n = 3, P < 0.001) and in cells stimulated with growth factors for 1 hour (46.5 ± 9.1 [stimulated] versus 4.6 ± 0.4 [rapamycin] and 7.7 ± 0.9 [wortmannin] pmol/min per milligram protein, P < 0.001; Figure 1A). Although FK506 alone had no influence on the growth factor–stimulated increase of p70 S6 kinase activity (48.2 ± 4.8 pmol/min per milligram protein, n = 3), the inhibitory effect of rapamycin on the activity of the enzyme was almost completely antagonized when excess concentrations of FK506 (10 μmol/L) were used to compete with rapamycin for the FKBP (40.9 ± 5.1 pmol/min per milligram protein), indicating that rapamycin exerted its inhibitory actions through mTOR. Furthermore, activation of p70 S6 kinase was accompanied by a strong shift toward slower migrating bands on SDS-PAGE, indicating hyperphosphorylation of the enzyme (Figure 1B). In quiescent CASMCs, addition of growth factors also induced a phosphorylation of 4E-BP1. Hyperphosphorylation of p70 S6 kinase and 4E-BP1 could efficiently be inhibited when the cells were pretreated with low nanomolar concentrations of rapamycin or wortmannin.

The inhibition of PI 3-kinase signaling pathway by either rapamycin or wortmannin was accompanied by a reduction, but not complete inhibition, of growth factor–induced cellular protein synthesis by 59% or 49%, respectively, as measured by [3H]leucine incorporation (for online Table I, please see www.ahajournals.org). Furthermore, [3H]thyidine incorporation, an indicator of DNA synthesis, was reduced by 68% or 73% when growth factor–stimulated CASMCs were treated with rapamycin or wortmannin, respectively. FK506 was able to reverse the inhibitory influence of rapamycin on protein and DNA synthesis.

These data suggest that proliferation of CASMCs is dependent on the integrity of the PI 3-kinase signaling pathway. Therefore, we investigated whether this signaling pathway is involved in the translation of cell cycle genes. Blockade of PI 3-kinase or mTOR by either wortmannin (10 nmol/L) or rapamycin (10 nmol/L), respectively, was associated with a marked inhibition of the mitogen-induced increases in the protein level of cyclin B1, cyclin D1, cyclin E, Cdk1, Cdk2, PCNA, and p21Cip1 (Figure 2). Protein levels of Cdk4, which does not oscillate, and of PI 3-kinase were used as housekeeping proteins. The increase in kinase activities of Cdk1 and Cdk2 were also prevented by either drug. The structurally distinct PI 3-kinase inhibitor LY294002 was similarly able to prevent the upregulation and activation of examined cell cycle proteins (data not shown).

RPA confirmed that no effect of these agents was observed on the growth factor–induced increase of their mRNA levels. The absence of an inhibitory effect on mRNA levels was verified by densitometric quantification normalized to housekeeping gene mRNA levels in each sample, further suggesting that these drugs inhibited cell cycle protein accumulation in a posttranslational manner.

**Figure 1.** Rapamycin (RPM) or wortmannin (WT) blocks growth factor–induced hyperphosphorylation and activation of p70 S6 kinase and 4E-BP1 in vitro. p70 S6 kinase activity was measured via immune complex kinase assay (A), whereas its hyperphosphorylation and hyperphosphorylation of 4E-BP1 (B) were determined by immunoblot in quiescent CASMCs, after stimulation for 1 hour with growth factors (GFs) alone or after preincubation with 10 nmol/L RPM or WT. FK506 (10 μmol/L) was used for competition experiments (n = 3). *P < 0.001 vs GF free; +P < 0.001 vs GF treatment.

**Figure 2.** Inhibition of PI 3-kinase signaling with RPM or WT prevents the GF-induced upregulation of the cell cycle proteins in CASMCs in vitro. Quiescent CASMCs were preincubated with RPM (10 nmol/L) or WT (10 nmol/L) for 30 minutes before GFs were added for a further 24 hours (fresh WT was given every 5 hours). Whole-cell lysates were used for immunoblotting (PI 3-kinase and Cdk4 were used as housekeeping proteins) and for immune histone H1 kinase assay.
In vitro data similar to those seen in human CASMCs were obtained with primary cultures of rat aortic smooth muscle cells (data not shown).

In Vivo Balloon Injury Is Accompanied by Increase of p70<sub>S6</sub> Kinase Activity, 4E-BP1 Phosphorylation, and Rapamycin-Sensitive Induction of Cell Cycle Protein Expression

We postulated that an altered pattern of cell cycle protein expression associated with arterial balloon injury in vivo also correlates with an activation of p70<sub>S6</sub> kinase and 4E-BP1 phosphorylation. Therefore, we balloon-injured the left carotid artery of untreated rats and of rats pretreated with rapamycin. The activity of p70<sub>S6</sub> kinase was found to be increased within 6 hours of injury, and maximal activity levels were reached at 24 hours (for online Figure I, please see www.ahajournals.org). Thereafter, the kinase activity of the enzyme gradually decreased, reaching control levels within 7 days after balloon injury. This increase was completely prevented when rats were pretreated with rapamycin (Figure 5A). In fact, rapamycin treatment inhibited p70<sub>S6</sub> kinase activity to a level below control levels. Balloon injury in untreated animals induced an upward shift to slower migrating bands, consistent with a hyperphosphorylation of p70<sub>S6</sub> kinase, which was completely prevented by rapamycin.

In addition to p70<sub>S6</sub> kinase activation, 4E-BP1 was found to be hyperphosphorylated in rapamycin-treated animals (Figure 5B). Hyperphosphorylation was still present after 4 days of injury, although an intermediate band reappeared, indicating a decrease of phosphorylation toward the uninjured control state. The hyperphosphorylational state of 4E-BP1 was abolished even below control levels when rats were pretreated with rapamycin.

The cell cycle proteins cyclin B1, cyclin D1, cyclin E, Cdk1, Cdk2, and PCNA, expressed at lowest levels in uninjured control arteries, were induced in response to injury within 24 hours in untreated animals, indicating the entry of vascular cells into the cell cycle (for online Figure II, please see www.ahajournals.org). A plateau was reached within 4 to 7 days after injury, with a later decline over a 3-week period (data not shown). p21<sub>Cip1</sub> expression was first detectable after 4 days of injury, although an intermediate band reappeared, indicating a decrease of phosphorylation toward the uninjured control state. The hyperphosphorylational state of 4E-BP1 was abolished even below control levels when rats were pretreated with rapamycin.

An important alternative pathway for rapamycin inhibition of cell cycle progression in other cell types, particular in T lymphocytes and murine BC3H1 cells, involves stabilization of p27<sub>Kip1</sub>. Although growth factor stimulation did induce a drop of p27<sub>Kip1</sub> protein levels in untreated CASMCs within 24 hours (Figure 4), this downregulation was not influenced by CASMC treatment with rapamycin. To confirm the validity of our assay, we reproduced the previously described stabilizing effect of rapamycin on p27<sub>Kip1</sub> levels in BC3H1 cells.

Figure 4. Growth factor–induced downregulation of the cyclin-dependent kinase inhibitor p27<sub>Kip1</sub> is not affected by inhibition of PI 3-kinase signaling pathway in primary cultures of CASMCs. Quiescent CASMCs and murine BC3H1 cells were GF-stimulated for 24 hours in the absence or presence of RPM (10 nmol/L). Cells were harvested, and whole-cell lysates were analyzed by immunoblot for p27<sub>Kip1</sub> protein levels.
immunoblot 4 days after balloon injury (7 days for p21 Cip1), the time of maximal expression in injured arteries of vehicle-treated animals (Figure 6 and online Figure II). As in CASMC culture, p27 Kip1 was found to be highly expressed in quiescent uninjured arteries of untreated animals, and p27 Kip1 protein levels were rapidly downregulated after balloon injury, reaching a nadir after 24 hours (online Figure II). Thereafter, the level increased over the following week, achieving baseline levels after 3 weeks (data not shown). However, as in CASMCs in vitro, the downregulation of p27 Kip1 24 hours after arterial balloon injury was not prevented by rapamycin treatment (Figure 6).

Discussion

Efforts at designing therapeutic manipulation of vascular cell cycle gene expression have focused primarily on the level of mRNA, such as antisense or transcription factor decoy oligodeoxynucleotides. However, not much is known about posttranscriptional regulation of cell cycle protein expression in vascular cells.

The data from the present study demonstrate that vascular injury in vivo leads to a rapid phosphorylation of components of the PI 3-kinase signaling, as has been observed after mitogenic stimulation of vascular smooth muscle cells (VSMCs) in vitro. Our data further suggest a critical role for translational regulation of cell cycle–regulatory protein expression in CASMCs. Neither rapamycin nor wortmannin influenced the growth factor–stimulated increase in mRNA levels of the cell cycle proteins studied, yet these agents reduced their protein levels. Therefore, we postulate that the PI 3-kinase signaling pathway is critical for increased translation of cell cycle–promoting proteins in vascular cells.

The results of the present study also suggest a striking difference between the response of VSMCs and of some other cell types to the drug rapamycin and, thereby, underscore the significance of translational regulation of cell cycle protein expression in VSMCs. Neither rapamycin nor wortmannin prevented the growth factor–induced or injury-induced downregulation of the cyclin-dependent kinase inhibitor p27 Kip1 in our primary culture of CASMCs, but both completely blocked the upregulation of cell cycle–promoting proteins. As previously shown by others, upregulation of the cyclin-dependent kinase inhibitor p21 Cip1 was prevented as well, further suggesting an alternative pathway of cell cycle inhibition. Similar in vitro results were obtained by using rat aortic smooth muscle cells. In T lymphocytes, rapamycin does inhibit mitogen-induced downregulation of p27 Kip1, thereby preventing the enzymatic activation of cyclin/Cdk complexes, but does not influence Cdk protein levels. Rapamycin has previously been shown to stabilize p27 Kip1 levels in murine BC3H1 cells, which closely resemble skeletal muscle cells, and we were able to confirm this obser-

Figure 5. In vivo treatment of rats with RPM suppresses injury-induced increase of p70 S6 kinase activity and 4E-BP1 phosphorylation. Rats were pretreated with RPM (6 mg/kg per day) or vehicle alone before balloon injury was induced. Treatment was continued until 1 day before the animals were euthanized. A, p70 S6 kinase activity was measured via immune complex kinase assay of whole-vessel homogenates (n=3, *P<0.001), and hyperphosphorylation of the enzyme was documented by immunoblot. B, 4E-BP1 protein was determined by immunoblot. CTRL denotes uninjured arteries of RPM-treated or vehicle-treated animals.

Figure 6. In vivo treatment with RPM abolishes the injury (INJ)-induced upregulation of cell cycle proteins. Rats were treated as described in Figure 5. The animals were euthanized 1 day (for RPM and p27 Kip1 immunoblot) and 4 days (for cyclin B1, cyclin D1, cyclin E, Cdk1, Cdk2, PCNA, and p21 Cip1 immunoblot) after balloon injury. CTRL denotes uninjured arteries of RPM-treated or vehicle-treated animals. PI3K and Cdk4 were used as housekeeping proteins for the 4-day treatment group.
In the present study, it was shown that inhibition of PI 3-kinase signaling in regulating the cell cycle in vascular cell systems in vitro or in vivo. Rapamycin has been shown to inhibit VSMC proliferation and migration in vitro and arterial intimal thickening after balloon injury in vivo.7, 27-29 Although Gallo et al.29 suggested a p27Kip1-stabilizing effect of rapamycin after vascular injury, they failed to examine p27Kip1 protein levels at critical early time points after injury. In a separate study of VSMCs in vitro, rapamycin inhibited Cdk1 and Cdk2 kinase activation without altering their protein levels.30 However, the subconfluent VSMCs and low serum conditions used in that study, unlike ours, do not result in downregulation of Cdk1 and Cdk2 protein levels and, therefore, do not require new Cdk protein synthesis on stimulation. Our contact-inhibited serum-withdrawn CASMCs achieved a strong up-regulation of p27Kip1 and a near complete downregulation of Cdk protein levels, closely resembling quiescent CASMCs of the normal uninjured artery, which also requires new Cdk protein synthesis for cell growth. Furthermore, the observed time course of p70S6 kinase activity after growth factor stimulation supports the previously described hypothesis that increased p70S6 kinase activity is required for entry of resting cells into the cell cycle but is not required by cells that are continuing to cycle.31

We verified our observations by using complementary pharmacological approaches to block the PI 3-kinase signaling pathway. Low nanomolar concentrations of wortmannin, as used in the present study, specifically inhibit PI 3-kinase, whereas much higher concentrations are required to inhibit either mitogen-activated protein kinase or even mTOR.35 The structurally unrelated PI 3-kinase inhibitor LY294002 also exerted an inhibitory effect (data not shown). PI 3-kinase is thought to mediate the majority of p70S6 kinase activation independently of mTOR. Of course, the impact of PI3-kinase inhibition on cell cycle progression is not limited to its effects on p70S6 kinase and 4E-BP1. For example, protein kinase B (Akt) may play a significant role. However, the inhibition of cell cycle protein upregulation seen after blockade either of PI3-kinase by wortmannin or of mTOR by rapamycin, despite accumulation of corresponding mRNAs, suggests a critical role for p70S6 kinase and 4E-BP1 in the translation of proteins required for cell cycle progression. An alternative explanation for the failure of wortmannin-treated cells to accumulate cyclin D1 protein is suggested by the work of Diehl et al.32 and others who have demonstrated a PI 3-kinase–dependent mechanism for inhibition of glycogen synthase kinase 3β-mediated degradation of cyclin D1. However, rapamycin inhibition of mTOR has not been found to suppress the reduction in glycogen synthase kinase 3 activity observed after treatment with insulin13, 34 (one of the growth factors used in the present study), nor can this mechanism explain the inhibition of the wide array of cell cycle proteins documented in the present study.

p70S6 kinase and eIF-4E promote translation of otherwise inefficiently translated mRNAs with highly structured 5′-untranslated regions,6 such as cell cycle–regulatory genes. Although targeted “knockout” of either 4E-BP1 or of p70S6 kinase has been reported,35 the cell cycle inhibition observed in the present study was associated with the simultaneous blockade of both of these complementary systems. The findings of the present study have intriguing implications for the design of cardiovascular therapeutics. Targeting the translational machinery may prevent not only the mitogen- or injury-induced upregulation of cell cycle proteins but also interrupt other pathophysiological events, such as cellular hypertrophy or cellular migration, during the development of vascular diseases.28, 36, 37 In fact, the postranscriptional regulation of protein expression may prove an important target for modifying the onset and progression of a wide range of disease processes.

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References


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