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Sepsis-induced suppression of skeletal muscle translation initiation mediated by tumor necrosis factor α

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Abstract

Inhibition of translational efficiency is responsible at least in part for the sepsis-induced decrease in protein synthesis observed in skeletal muscle. Moreover, infusion of the inflammatory cytokine tumor necrosis factor α (TNF-α) into naive rats produces a comparable decrement. Therefore, the purpose of the present study was to determine whether inhibition of TNF action under in vivo conditions could prevent the sepsis-induced decrease in translation initiation observed in the postabsorptive state. To address this aim, sepsis was produced by cecal ligation and puncture (CLP) and rats were studied in the fasted condition 20 to 24 hours thereafter. Both septic and time-matched nonseptic control rats were pretreated with TNF-binding protein (TNF_{BP}) before CLP or sham surgery to neutralize endogenously produced TNF. Sepsis altered the distribution of eukaryotic initiation factor 4E (eIF4E) in the gastrocnemius by increasing the amount associated with 4E-BP1 (inactive complex) and decreasing the amount bound to eIF4G (active complex). This change in eIF4E availability was associated with a decreased phosphorylation of 4E-BP1. Furthermore, the phosphorylation of ribosomal protein S6 and mammalian target of rapamycin (mTOR) was also decreased in the gastrocnemius from septic rats. Pretreatment of septic rats with TNF_{BP} largely ameliorated the altered distribution of eIF4E as well as the reduced phosphorylation of 4E-BP1, S6, and mTOR. In contrast, sepsis did not change either the total amount or the phosphorylation state of eIF2α or eIF2Bε. Furthermore, no sepsis-induced change in eIFs was detected in the slow-twitch soleus muscle. The ability of TNF_{BP} to prevent the sepsis-induced alterations in translation initiation was independent of change in plasma insulin and proportional to the insulinlike growth factor I content in blood and muscle but was associated with a reduction in plasma corticosterone. Hence, the decreased constitutive protein synthesis observed in fast-twitch skeletal muscle in response to peritonitis is mediated by a TNF-dependent mechanism affecting mTOR regulation of translation initiation.

1. Introduction

Negative nitrogen balance and the loss of muscle protein are hallmarks of gram-negative sepsis [1]. The erosion of lean body mass in this condition results from both a decrease in muscle protein synthesis and an increase in proteolysis [2-5]. Previous work in animal models of sepsis indicates the impairment of protein synthesis occurs in muscles with a predominance of fast-twitch fibers (eg, gastrocnemius) as opposed to slow-twitch fibers (eg, soleus) [5]. Moreover, the sepsis-induced inhibition of protein synthesis primarily results from a decrease in translational efficiency, rather than a decrease in number of ribosomes [5,6].

The process of messenger RNA (mRNA) translation involves 3 steps: initiation, elongation, and termination. In general, impaired muscle protein synthesis induced by hypermetabolic sepsis in rats results from multiple defects in the initiation phase of translation [7]. Initiation is regulated by a large number of protein factors, termed eukaryotic initiation factors (eIFs). One of these initiation factors, eIF2, mediates the first step in initiation, which involves the attachment of the initiator methionyl-transfer ribonucleic acid (tRNA) (met-$tRNA_i^{met}$) to the 40S ribosomal subunit to form the 43S preinitiation complex [8]. Moreover, the activity of eIF2 can be modulated by the activity of another initiation factor, eIF2B [9]. A decrease in the amount of catalytic ε subunit of eIF2B has been reported in some models of chronic peritonitis [10,11].

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A second regulatory step in initiation involves the binding of mRNA to the 43S preinitiation complex that is mediated by eIF4F [12]. The stable ternary complex referred to as eIF4F plays a pivotal role in controlling peptide-chain initiation by regulating the recruitment of the 43S preinitiation complex to the mRNA. The eIF4F complex is heterotrimeric (eg, eIF-4E, -4G, and -4A) and, of these subunits, eIF4E is considered rate limiting in the binding of mRNA to ribosomes [13]. eIF4E has high affinity for the m^7GTP cap structure present at the 5′ end of all nuclear transcribed mRNAs and is necessary for formation of the eIF4E · mRNA complex. During translation initiation, the eIF4E · mRNA complex interacts with eIF4G and eIF4A to form the active eIF4F holoenzyme, thereby allowing cap-dependent translation to proceed. In muscle, the interaction between eIF4E and the scaffold protein eIF4G is controlled in part by the eIF-4E binding protein 1 (BP-1) that functions as a cap-dependent translational repressor [14]. This binding protein obstructs the interaction of eIF4G with eIF4E and thereby limits the assembly of the active eIF4F complex. The increased phosphorylation of 4E-BP1 releases it from eIF4E and consequently facilitates the binding of eIF4E with eIF4G. Both sepsis and endotoxemia decrease constitutive 4E-BP1 phosphorylation in skeletal muscle [3,15,16], but the physiologic mechanism producing this change remains poorly understood.

Regulation of mRNA translation is also exerted by the multisite phosphorylation and activation of the serine (Ser)/threonine (Thr) protein kinase S6K1 [17]. This activation may play a role in the translational regulation of the mRNA family that encodes proteins containing a terminal oligopyrimidine (TOP, eg, 5′-TOP) tract downstream of their transcription initiation site [18], although this remains an area of controversy [19,20]. Currently, the mechanism by which sepsis decreases the phosphorylation of S6K1 and its physiologic downstream substrate, ribosomal protein (rp) S6, remains unknown.

The proinflammatory cytokine tumor necrosis factor α (TNF-α) is synthesized and secreted by numerous cell types and tissues in response to bacterial infection or lipopolysaccharide (endotoxin). Cytokines can potentially modulate cellular functions either locally in an autocrine/paracrine manner or at sites distant from their origin of synthesis in a manner consistent with that of endocrine hormones. Although blood-borne cytokines are primarily derived from macrophage-rich tissues, nonimmune tissues (eg, striated muscle) also synthesize TNF-α and other inflammatory cytokines [21,22]. Hence, an elevation in the circulating or tissue concentration of TNF-α has been posited to alter a wide array of metabolic functions. In this regard, exogenous TNF-α both increases muscle proteolysis [23] and decreases muscle protein synthesis and selected aspects of mRNA translation [24]. Conversely, pretreatment of rats with agents that decrease circulating TNF-α have been reported to improve muscle protein balance in septic rats by preventing either the elevated rate of proteolysis or the decreased rate of muscle protein synthesis [23,25-27], although the underlying mechanisms for these changes are not fully elucidated. Therefore, the purpose of the present study was to focus on the synthetic side of the protein balance equation by assessing selected regulatory elements that control translation initiation and protein synthesis. To define the cellular mechanism by which endogenous TNF-α impairs muscle protein synthesis, septic and time-matched control rats were pretreated with TNF-binding protein (TNF_{BP}), and selected regulatory steps controlling peptide-chain initiation were assessed in both slow- and fast-twitch skeletal muscle.

2. Materials and methods

2.1. Animals and experimental protocols

Male Sprague-Dawley rats weighing 200 to 225 g were purchased from Charles River Breeding Laboratories (Cambridge, MA). Rats were acclimated for 1 week in a light-controlled room (12 hours light/12 hours dark cycle) under constant temperature. Water and standard rat chow were provided ad libitum. All experiments were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine and adhered to the National Institutes of Health (NIH) guidelines for the use of experimental animals.

Rats were injected subcutaneously with TNF_{BP} (1 mg/kg, 1 mL/rat; Amgen, Boulder, CO) to determine the potential role of endogenously produced TNF-α as a mediator of the sepsis-induced changes in signal transduction. TNF_{BP} is a dimeric, polyethylene glycol–linked form of the human p55 soluble TNF receptor that antagonizes the actions of TNF-α [28]. The timing and dose of the synthetic TNF receptor are based on previous work demonstrating its effectiveness in preventing the sepsis-induced loss of skeletal muscle protein mass [25]. Four hours after TNF_{BP} administration, rats were anesthetized with pentobarbital sodium (50-60 mg/kg), a midline laparotomy was performed, and sepsis was produced by cecal ligation and puncture (CLP). The cecum was ligated at its base and punctured twice with a 20-g needle, returned to the peritoneal cavity, and the muscle and skin layers were individually closed. Rats were resuscitated with 10 mL of 0.9% sterile saline administered subcutaneously. Time-matched nonseptic control animals were subjected to a midline laparotomy with intestinal manipulation, and resuscitated with the same volume of saline. During the operation, rats were placed on a warming pad to maintain body temperature. After surgery, food was withheld but the animals were permitted free access to water. Hence, observed changes between septic and nonseptic rats cannot be attributed to differences in food intake or nutritional status. Twenty to 24 hours after CLP, rats were anesthetized and a blood sample was collected from the abdominal aorta into a heparinized syringe. Thereafter, skeletal muscle (gastrocnemius and soleus) was excised and a portion homogenized,

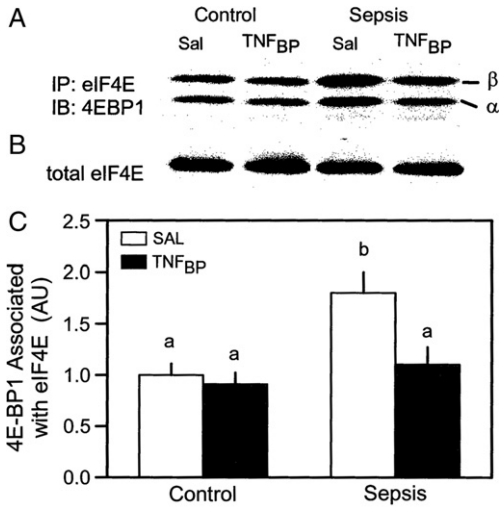

Fig. 1. Sepsis-induced changes in the binding of 4E-BP1 with eIF4E in the gastrocnemius muscle. Rats were subjected to CLP to induce sepsis and studied approximately 18 to 20 hours thereafter; nonseptic control rats underwent sham surgery and were time-matched and pair-fed. Four hours before CLP, rats were injected subcutaneously with either TNF_{BP} or an equal volume of saline (1 mL). A, eIF4E was immunoprecipitated (IP) and the amount of 4E-BP1 bound to eIF4E assessed by immunoblotting (IB); the positions of the α- and β-isoforms are so indicated. B, Representative Western blot of total eIF4E demonstrating no differences among groups. C, Bar graph represents densitometric analysis of immunoblots of 4E-BP1 associated with eIF4E, where the value from the control + saline (Sal) group was set at 1.0 AU. Values are means ± SEM; n = 9 to 10 rats per group. Means with different letters are statistically different from each other ($P < .05$).

and the remaining tissue was freeze-clamped. All blood and tissue samples were stored at −70°C until analyzed.

2.2. Immunoblotting and immunoprecipitation

After immunoprecipitation of 4E-BP1 from tissue homogenates, the various phosphorylated forms of 4E-BP1 were separated by sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) and analyzed by protein immunoblotting [3,15,16,24]. Other Western blots were performed using either primary antibodies to total S6K1 (no. 230, Santa Cruz Biotechnology, Santa Cruz, CA), total 4E-BP1 (Bethyl Laboratories, Montgomery, TX), phosphospecific 4E-BP1 (Thr37; Cell Signaling Technology, Beverly, MA), total and phosphorylated (Ser 2448 and Ser2481) mammalian target of rapamycin (mTOR, Bethyl Laboratories), total and phosphorylated S6 (Ser235/Ser236; Cell Signaling), total and phosphorylated eIF2α (Ser51; Cell Signaling), or total and phosphorylated eIF2Bε (Ser35; Cell Signaling). The blots were developed by using enhanced chemiluminescence (Amersham, Pharmacia Biotech, Piscataway, NJ). The blots were exposed to x-ray film in a cassette equipped with a DuPont (Boston, MA) Lightning Plus intensifying screen. After development, films were scanned (Microtek ScanMaker IV, Carson, CA) and analyzed using NIH Image 1.6 software (NIH, Bethesda, MD).

The association of eIF4E with either 4E-BP1 or eIF4G was determined as previously described [16,24]. Briefly, fresh skeletal muscle was homogenized and eIF4E as well as 4E-BP1·eIF4E and eIF4G·eIF4E complexes were immunoprecipitated from aliquots of 10 000*g* supernatants by using an anti-eIF4E monoclonal antibody. The antibody-antigen complex was collected by incubation with BioMag goat antimouse IgG beads (Perseptive Biosystems, Framingham, MA). The beads were collected by centrifugation and the supernatants were subjected to electrophoresis either on a 7.5% polyacrylamide gel for analysis of eIF4G or on a 15% gel to quantify 4E-BP1 and eIF4E. Proteins were then electrophoretically transferred to nitrocellulose. The membranes were incubated with a mouse antihuman eIF4E antibody, a rabbit antirat 4E-BP1 antibody, or a rabbit anti-eIF4G antibody (Bethyl Laboratories). Autoradiographs were scanned and quantified as described above.

2.3. Isolation of mRNA, nuclease protection assay, and Northern blotting

Total RNA was extracted from the gastrocnemius only in a mixture of phenol and guanidine thiocyanate (TRI Reagent, Molecular Research Center, Cincinnati, OH) using the manufacturer's protocol. RNA was separated from protein and DNA by the addition of bromocholoropropane and precipitation in isopropanol. After a 75% ethanol wash and resuspension in formamide, RNA samples were quantified by spectrophotometry. Ten micrograms of RNA was used for each assay. TNF-α was determined by a commercial multiprobe rat template set (rCK-1; Pharmingen, San Diego, CA) with an in vitro transcription kit (Pharmingen). The labeled riboprobe was hybridized with RNA overnight by using a ribonuclease protection assay (RPA) and the manufacturer's protocol (Pharmingen). Protected RNAs were separated by a 5% acrylamide gel (19:1 acrylamide/bisacrylamide). Gels were transferred to blotting paper and dried under vacuum on a gel dryer. Dried gels were exposed to a phosphorimager screen (Molecular Dynamics, Sunnyvale, CA) and the resulting data were quantified with ImageQuant software and normalized to the rat ribosomal protein L32 mRNA signal in each lane, as previously described [21]. Insulin-like growth factor-I (IGF-I) mRNA content was determined by Northern blotting by using capillary transfer to Zeta-Probe GT blotting membranes (Bio-Rad Laboratories, Hercules, CA). An 800–base pair probe from rat IGF-I (Peter Rotwein, Portland, OR) was labeled by a Random Primed DNA Labeling kit (Roche Molecular Biochemicals, Indianapolis, IN) as previously described [15]. For normalization of RNA loading, a rat 18S oligonucleotide was radioactively end labeled with polynucleotide kinase (Amersham), and blots were stripped, reprobed, and quantified as described above.

2.4. Plasma determinations

Blood was centrifuged and the plasma concentrations of insulin (Linco Research, St Charles, MO), IGF-I (DSL, Webster, TX) and corticosterone (DSL) were measured by commercial radioimmunoassays.

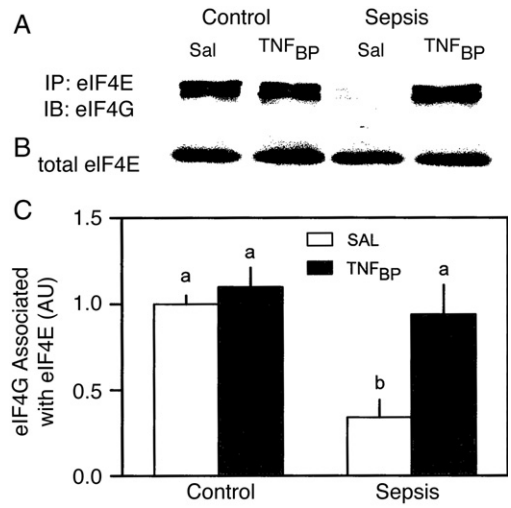

Fig. 2. Sepsis-induced changes in the binding of eIF4G with eIF4E in the gastrocnemius muscle. Rats were pretreated with either TNF_{BP} or saline 4 hours before CLP or sham surgery, and muscle samples were obtained 18 to 20 hours thereafter. A, eIF4E was immunoprecipitated (IP) and the amount of eIF4G bound to eIF4E assessed by immunoblotting (IB). B, Representative Western blot of total eIF4E demonstrating no differences between groups. C, Bar graph represents densitometric analysis of immunoblots of eIF4G associated with eIF4E, where the value from the control + saline (Sal) group was set at 1.0 AU. Values are means ± SEM; n = 9 to 10 rats per group. Means with different letters are statistically different from each other ($P < .05$).

2.5. Statistical analysis

Data were obtained from separate experimental series each containing all 4 groups. Experimental values are presented as means ± SE. The number of rats in each group is indicated in the figure and table legends. Statistical evaluation of the data was performed by analysis of variance followed by the Student-Newman-Keuls test to determine treatment effect (Instat, San Diego, CA). Differences between the groups were considered significant at P less than .05.

3. Results

3.1. Sepsis-induced alterations in eIF4E availability

The mechanistic interactions between sepsis and TNF-α were investigated under in vivo conditions by analyzing known regulatory steps in the control of translation initiation. To this end, the extent of the functional eIF4F complex was first assessed. Fig. 1 illustrates that the α- and β-isoforms of 4E-BP1 were detected in eIF4E immunoprecipitates of the gastrocnemius and the content of the inactive eIF4E · 4E-BP1 complex was increased approximately 80% compared with control values from time-matched nonseptic rats. When eIF4E is bound to 4E-BP1 it is unable to interact with eIF4G to form the active eIF4E · eIF4G complex. Fig. 2 illustrates under basal conditions the amount of active eIF4E · eIF4G complex was reciprocally reduced approximately 65% by the septic insult, compared with values from nonseptic control animals. Pretreatment of control rats with TNF_{BP} did not significantly alter eIF4E distribution between inactive and active complexes in the gastrocnemius from control rats. In contrast, TNF_{BP} essentially completely prevented the sepsis-induced redistribution of eIF4E. Hence, the amount of eIF4E · 4E-BP1 and eIF4E · eIF4G complexes in the gastrocnemius from the TNF_{BP} + sepsis group was not different from nonseptic control values (Figs. 1 and 2).

Many catabolic conditions lead to the redistribution of eIF4E between the active and inactive eIF4F complex via the actions of the translational repressor molecule 4E-BP1 [12,15,16,24,29]. Hyperphosphorylation of 4E-BP1 decreases the association of the binding protein with eIF4E and generally increases translation [14]. One-dimensional SDS-PAGE allows the various phosphorylated forms of 4E-BP1 to be resolved into 3 bands. Fig. 3A illustrates the amount of 4E-BP1 in the hyperphosphorylated γ form was down-regulated by sepsis and this decrease was prevented by TNF_{BP}. Comparable results were obtained when a phosphospecific antibody was used to detect changes in the phosphorylation state of Thr37/Thr46 in 4E-BP1 (Fig. 3B and C).

3.2. Sepsis-induced changes in S6K1, rpS6, and mTOR

S6K1 also resolves into multiple bands when analyzed by SDS-PAGE because variable phosphorylation yields differences in electrophoretic mobilities [17,18]. Hence,

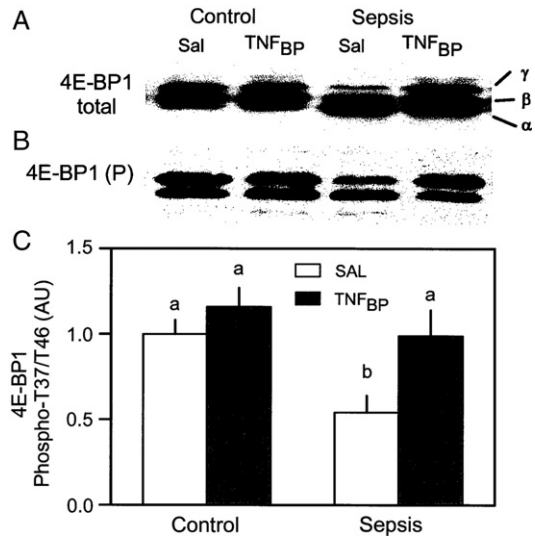

Fig. 3. Sepsis-induced changes in the phosphorylation of 4E-BP1 in the gastrocnemius muscle. Rats were pretreated with either TNF_{BP} or saline 4 hours before CLP or sham surgery, and muscle samples were obtained 18 to 20 hours thereafter. A, Representative immunoblot for total 4E-BP1 with the positions of the α-, β-, and γ-isoforms indicated; quantitation of all 3 bands indicated there was no sepsis-induced change in total 4E-BP1 (data not shown). B, Representative immunoblot of phosphorylated (P) 4E-BP1 using a phosphospecific antibody to T37/T46. C, Bar graph represents densitometric analysis of Thr37 phosphorylation, where the value from control + saline (Sal) group was set at 1.0 AU. Values are means ± SEM; n = 9 to 10 rats per group. Means with different letters are statistically different from each other ($P < .05$).

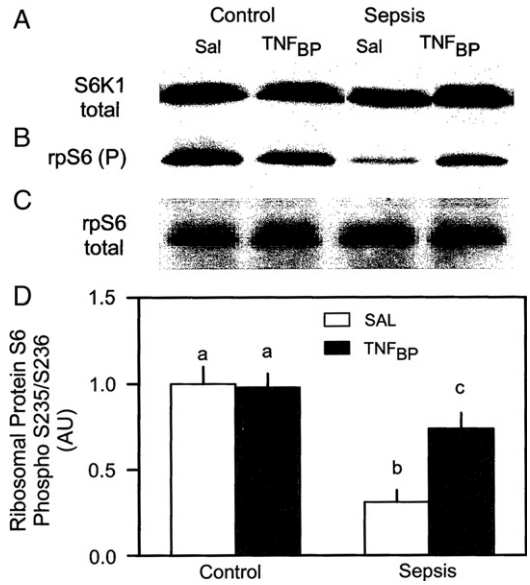

Fig. 4. Sepsis-induced changes in the phosphorylation of S6K1 and the ribosomal protein (rp) S6 in the gastrocnemius muscle. Rats were pretreated with either TNF_{BP} or saline 4 hours before CLP or sham surgery, and muscle samples were obtained 18 to 20 hours thereafter. A, Representative immunoblot for total S6K1. B and C, Representative immunoblots of phosphorylated (P) and total rpS6, respectively. D, Bar graph represents densitometric analysis of rpS6 phosphorylation where the value from the control + saline (Sal) group was set at 1.0 AU. Values are means ± SEM; n = 9 to 10 rats per group. Means with different letters are statistically different from each other ($P < .05$).

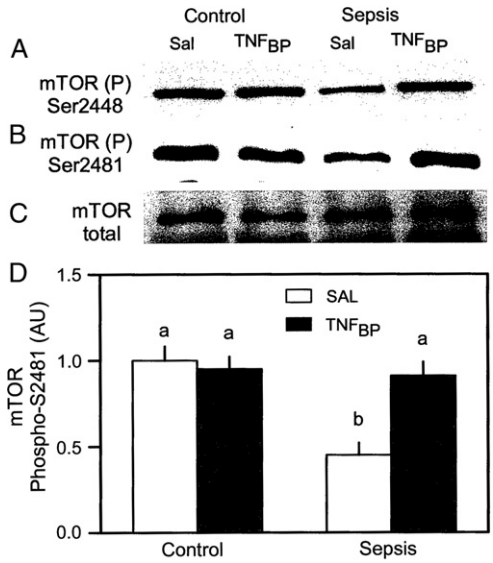

Fig. 5. Sepsis-induced changes of mTOR in the gastrocnemius muscle. Rats were pretreated with either TNF_{BP} or saline 4 hours before CLP or sham surgery, and muscle samples were obtained 18 to 20 hours thereafter. A, B, and C, Representative immunoblots of Ser2448-phosphorylated (P), Ser2481-phosphorylated, and total mTOR, respectively. D, Bar graph represents densitometric analysis of Ser2481 phosphorylated mTOR where the value from the control + saline (Sal) group was set at 1.0 AU. Values are means ± SEM; n = 9 to 10 rats per group. Means with different letters are statistically different from each other ($P < .05$).

the most slowly migrating forms of S6K1 represent the heavily phosphorylated and most activated form of the kinase. In the gastrocnemius from control rats, there was modest constitutive S6K1 phosphorylation (Fig. 4A). Sepsis alone appeared to increase the mobility of the top band, indicating a relative dephosphorylation of S6K1. In septic rats pretreated with TNF_{BP}, the mobility of the top band decreased back to control values. The S6K1 immunoblot was not quantified because the 2 bands could not be adequately separated. However, the phosphorylation status of the ribosomal protein S6, which is often used as a surrogate marker of S6K1 activity, was determined and quantified. Use of a phosphospecific antibody directed against the first and second phosphorylation sites, Ser236 and Ser235 [30], showed that sepsis clearly produced a dephosphorylation of rpS6 in the gastrocnemius (Fig. 4B). Furthermore, administration of TNF_{BP} prevented the reduction in constitutive rpS6 phosphorylation. Neither the sepsis- nor TNF_{BP}-induced change in S6 resulted from a change in total S6 protein (Fig. 4C and D).

The proline-directed Ser/Thr protein kinase referred to as mTOR is believed to be a common upstream mediator and is responsible, at least in part, for the phosphorylation of 4E-BP1 and S6K1 [29]. Sepsis consistently decreased the phosphorylation of mTOR on Ser2481 and Ser2448 by 45% to 55% and this response was prevented by TNF_{BP} (Fig. 5A, B, and D). Neither sepsis nor TNF_{BP} altered total mTOR content in muscle (Fig. 5B).

3.3. Soleus muscle

Sepsis preferentially decreases protein synthesis in muscle with a predominance of fast-twitch fibers (eg, gastrocnemius) [5]. Therefore, as a "negative control" various regulatory elements of translation initiation were also assessed in the slow-twitch soleus. We detected no statistically significant changes in the distribution of eIF4E or the phosphorylation of 4E-BP1, rpS6, or mTOR in soleus muscle from septic rats compared with values from time-matched nonseptic control rats (data not shown).

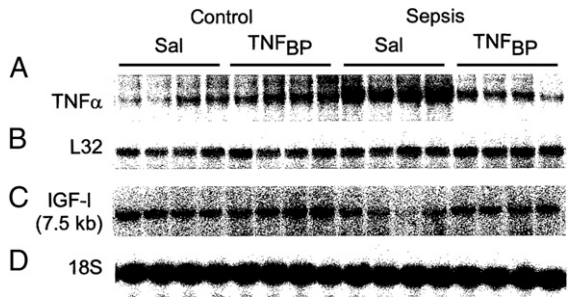

Fig. 6. Sepsis-induced changes in the content of TNF-α and IGF-I mRNA in the gastrocnemius muscle. Rats were pretreated with either TNF_{BP} or saline 4 hours before CLP or sham surgery, and muscle samples were obtained 18 to 20 hours thereafter. A and B, Representative autoradiographs from a nuclease protection assay for TNF-α and L32, the latter of which was used as a loading control. C and D, Representative Northern blots of the major 7.5-kilobase IGF-I mRNA transcript and 18S loading control. Representative blots show an n = 4 for each group, but are representative of 9 to 10 rats per group.

Table 1
Effect of sepsis on plasma hormone concentrations

	Saline + nonseptic	TNF_{BP} + nonseptic	Saline + sepsis	TNF_{BP} + sepsis
Insulin (pmol/L)	102 ± 14[a]	113 ± 15[a]	119 ± 18[a]	107 ± 16[a]
IGF-I (ng/mL)	547 ± 41[a]	560 ± 43[a]	372 ± 33[b]	511 ± 35[a]
Corticosterone (ng/mL)	171 ± 18[a]	159 ± 27[a]	447 ± 36[b]	275 ± 31[c]

Values are means ± SEM; n = 9 to 10 rats per group. Means with different letters for a specific parameter are statistically different from each other ($P < .05$). Rats were pretreated with either TNF_{BP} or saline 4 hours before CLP or sham surgery, and blood samples were obtained 24 hours thereafter.

3.4. Sepsis-induced changes in the eIF2/eIF2B system

Sepsis induced by CLP did not significantly alter either the total amount of eIF2α or eIF2Bε, or the phosphorylation state of these 2 regulatory factors in the gastrocnemius at the time point examined (data not shown). In addition, no change in the total amount or phosphorylation state of eIF2α and eIF2Bε was detected in muscle from either nonseptic or septic rats treated with TNF_{BP} (data not shown).

3.5. Tissue TNF-α and IGF-I mRNA content

Muscle from septic rats showed a 3- to 7-fold increase in the mRNA content for TNF-α when sampled 20 to 24 hours after CLP, compared with values from control rats (Fig. 6A). In addition, sepsis decreased IGF-I mRNA by 35% (Fig. 6C). Both of these sepsis-induced changes, which can potentially regulate protein synthesis either directly or indirectly, were prevented by TNF_{BP}.

3.6. Plasma hormone concentrations

The plasma insulin concentration was not significantly altered by either sepsis or the administration of TNF_{BP} (Table 1). In contrast, sepsis reduced the plasma IGF-I concentration by 32% and this decrease was prevented by TNF_{BP} (Table 1). Sepsis also more than doubled the plasma corticosterone concentration, compared with control values (Table 1). Treatment of rats with TNF_{BP} reduced the sepsis-induced increase in corticosterone by approximately 60% but did not alter corticosterone in control rats.

4. Discussion

When protracted, numerous forms of stress lead to the erosion of lean body mass [1]. Sepsis, endotoxin, and inflammatory cytokines have all been reported to modulate muscle protein balance, at least in part, by decreasing global protein synthesis [3-5,15,24]. This reduction is characterized by an impaired synthetic rate of both the sacroplasmic and myofibrillar protein pools in muscle from septic or TNF-α–infused rats [6,24]. Previous studies have reported sepsis-induced muscle atrophy can be prevented or attenuated by agents that either neutralize circulating TNF-α or prevent its synthesis [25,27,31]. Furthermore, pretreatment of septic rats with TNF_{BP} prevents the characteristic reduction in global protein synthesis [25]. The current studies advance a mechanistic understanding of the protein synthetic response by demonstrating that TNF_{BP} prevents sepsis-induced defects in mTOR signaling. mTOR represents an up stream signaling component and a bifurcation point for the regulation of 4E-BP1 and S6K1/S6 phosphorylation [32]. We assessed the phosphorylation of mTOR at 2 sites—Ser2481 and Ser2448. Phosphorylation of the first site is indicative of autophosphorylation of the protein and considered crucial in the activation of the kinase, whereas phosphorylation of the second site appears mediated by several kinases, including PKB and S6K1, under different experimental conditions [33,34]. Our current results, demonstrate a coordinated decrease in mTOR phosphorylation at both Ser2481 and Ser2448, and therefore suggest sepsis diminished mTOR kinase activity. Pretreatment of septic rats with TNF_{BP} prevented the reduction in mTOR phosphorylation. In response to nutrient deprivation and growth factor stimulation, the kinase activity of mTOR can be regulated by physical interactions with several newly reported proteins, such as raptor, rictor, and GβL [35]. Although not directly assessed in the current study, it seems likely that TNF-α, either directly or indirectly, alters one or more of these mTOR-protein interactions thereby impairing translation initiation and muscle protein synthesis.

As mentioned above, growth factors such as insulin and IGF-I enhance mTOR phosphorylation and thereby stimulate protein synthesis [12,32]. In this regard, the ability of TNF_{BP} to prevent the sepsis-induced decrease in mTOR phosphorylation could not be attributed to a concomitant change in the plasma insulin concentration. In contrast, changes in phosphorylated mTOR in response to sepsis and/or TNF_{BP} were inversely proportional to changes in the IGF-I content of plasma and muscle. Although such an association does not prove causality, these data are consistent with the known anabolic effects of IGF-I in skeletal muscle that are mTOR-dependent [32]. These results are consistent with previously published studies showing a reduction in muscle IGF-I mRNA in response to TNF-α under both in vivo conditions and in cultured myocytes [24,36]. Moreover, the data are consistent with the ability of TNF-neutralizing agents to prevent the decrease in plasma IGF-I seen in sterile inflammation produced by zymosan [37] and the sepsis-induced growth hormone resistance [38]. Finally, TNF_{BP} also prevented the sepsis-induced increase in muscle TNF-α mRNA content, although the mechanism for this change was not specifically investigated.

One caveat of in vivo studies in general and the current study in particular is the inability to determine whether endogenously produced TNF-α was directly or indirectly responsible for the sepsis-induced decrease in mTOR phosphorylation and its downstream effects. There is some controversy regarding whether TNF-α can directly alter muscle protein synthesis. For example, several previous studies have failed to detect a consistent reduction in the in

vivo rate of muscle protein synthesis [23,39-41]. However, these studies were limited by the transient elevation in TNF-α produced by a bolus injection of cytokine, and when TNF-α was continuously administered for at least 24 hours, a decrease in total and sacroplasmic protein synthesis as well as a decrease in translation initiation was detected [24,42]. Likewise, there are conflicting data on the ability of TNF-α to directly inhibit muscle protein synthesis under in vitro and in situ conditions [43-46]. Hence, whether the increased production of endogenous TNF-α is directly responsible for our current finding pertaining to changes in the protein synthesis signal transduction pathway remains equivocal. However, it is noteworthy that TNF_{BP} also significantly reduced the sepsis-induced increase in circulating corticosterone. Excess glucocorticoids also impair mTOR-mediated muscle protein synthesis and produce muscle wasting [12,47,48]. Therefore, in the present study we cannot exclude the possibility of an indirect effect of TNF-α on translation initiation mediated by a secondary increase in corticosterone. Such an indirect effect has been previously reported for the endotoxin-induced decrease in muscle IGF-I [49] and the sepsis-induced increase in muscle ubiquitin-proteasome–dependent proteolysis [50].

Increased mTOR kinase activity stimulates the phosphorylation of 4E-BP1 and S6K1, whereas rapamycin-induced inhibition of mTOR produces a reciprocal decrease in the phosphorylation of these intermediates controlling translation initiation [32]. In the current study, the hypermetabolic polymicrobial septic insult decreased basal phosphorylation of the translational repressor molecule 4E-BP1. This change was associated with the redistribution of eIF4E from the active eIF4G · eIF4E complex to the inactive 4E-BP1 · eIF4E complex. Although protein synthesis was not directly determined in the present study, the ability of the eIF4E · eIF4G complex to bind to the mRNA cap has been reported to be a rate-limiting determinant of total protein synthesis in myocytes [51]. Comparable changes in the formation of the active eIF4F complex in muscle have also been reported in response to endotoxin [15], a condition characterized by excessive production of TNF-α, and by TNF-α per se [24]. Sepsis also decreased the constitutive phosphorylation of rpS6, which is a physiologic substrate for S6K1 and provides an indirect index of S6K1 activity [12,30]. The phosphorylation of S6K1 and S6 are associated with accelerated rates of mRNA translation initiation and a stimulation of skeletal muscle protein synthesis under in vivo conditions where other components of the translational apparatus have not been overexpressed or altered as a compensatory response to their deletion [12,16,29]. Although activation of S6K1 and the phosphorylation of rpS6 was originally believed to be the principle mechanism through which the translation of 5′ -TOP mRNAs was enhanced [52], recent studies indicate redundancy may exist between S6K1, S6K2, and a mitogen-activated protein kinase–dependent kinase in the mitogen-stimulated phosphorylation of rpS6 [19,20]. Therefore, the physiologic significance of the sepsis-induced decrease in S6K1 and S6 under in vivo conditions remains to be determined but is consistent with a defect in more proximal signal transduction elements. Pretreatment of rats with TNF_{BP} before CLP prevented the sepsis-induced decrease in 4E-BP1 and S6K1/S6 phosphorylation as well as the redistribution of eIF4E into the inactive complex. All of these changes are consistent with the above-mentioned ability of TNF_{BP} to prevent sepsis-induced decreases in mTOR kinase activity.

Finally, our data also suggest that 20 to 24 hours after CLP, the ability of eIF2 to form a ternary complex with GTP and met-$tRNA_i^{met}$, a potentially rate controlling step in translation initiation [8,9], was not decreased. Specifically, there was no sepsis-induced decrease in either the total amount or the phosphorylation states of either eIF2B or eIF2 in skeletal muscle. These results differ from those observed in a chronic (5 day) abscess model of sepsis, where a consistent decrease in the amount of eIF2Bε protein and mRNA expression has been reported [10,11]. The simplest explanation for the difference between the current CLP model and the abscess model relates to the more protracted duration of the insult in the latter condition. In this regard, eIF2Bε is only slightly decreased at 3 days postinfection, whereas it is substantially reduced (40%) at 5 days postinfection [10]. Therefore, collectively, these data indicate changes in the eIF2/2B system are not primarily responsible for the acute decrease in muscle protein synthesis produced in response to infection.

In summary, the results of the present investigation provide evidence that sepsis inhibits mTOR signaling pathways in fast-twitch, but not slow-twitch, skeletal muscle under postabsorptive in vivo conditions. This defect is evidenced by a reduced phosphorylation of 4E-BP1 that is associated with a redistribution of eIF4E from the active eIF4E · eIF4G complex to the inactive eIF4E · 4E-BP1 complex, and a reduced phosphorylation of rpS6. These sepsis-induced defects in skeletal muscle appear mediated, either directly or indirectly, by the endogenous overproduction of TNF-α. Furthermore, because TNF_{BP} also partially prevented the sepsis-induced increase in corticosterone, we cannot exclude the possibility that a reduction in this catabolic hormone was in part responsible for observed changes in the translational control of muscle protein synthesis.

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References

[1] Rennie MJ. Muscle protein turnover and the wasting due to injury and disease. Br Med Bull 1985;41:257-64.

[2] Hasselgren PO, James JH, Benson DW, Hall-Angeras M, Angeras U, Hiymam DT, et al. Total and myofibrillar protein breakdown in different types of rat skeletal muscle: effect of sepsis and regulation by insulin. Metabolism 1989;38:634-40.

[3] Svanberg E, Frost RA, Lang CH, Isgaard J, Jefferson LS, Kimball SR, et al. IGF-I/IGFBP-3 binary complex modulates sepsis-induced inhibition of protein synthesis in skeletal muscle. Am J Physiol Endocrinol Metab 2000;279:E1145-58.

[4] Vary TC, Dardevet D, Grizard J, Voisin L, Buffiere C, Denis P, et al. Differential regulation of skeletal muscle protein turnover by insulin and IGF-I after bacteremia. Am J Physiol Endocrinol Metab 1998; 275:E584-93.

[5] Vary TC, Kimball SR. Sepsis-induced changes in protein synthesis: differential effects on fast- and slow-twitch muscles. Am J Physiol Cell Physiol 1992;262:C1513-9.

[6] Vary TC, Owens EL, Beers JK, Verner K, Cooney RN. Sepsis inhibits synthesis of myofibrillar and sarcoplasmic proteins: modulation by interleukin-1 receptor antagonist. Shock 2000;279:13-8.

[7] Cooney RN, Kimball SR, Vary TC. Regulation of skeletal muscle protein turnover during sepsis: mechanisms and mediators. Shock 1997;7:1-16.

[8] Proud CG. eIF2 and the control of cell physiology. Semin Cell Dev Biol 2005;16:3-12.

[9] Pavitt GD. eIF2B, a mediator of general and gene-specific translational control. Biochem Soc Trans 2005;33:1487-92.

[10] Vary TC, Jurasinski CV, Karinch AM, Kimball SR. Regulation of eukaryotic initiation factor-2 expression during sepsis. Am J Physiol Endocrinol Metab 1994;266:E193-E201.

[11] Voisin L, Gray K, Flowers K, Kimball SR, Jefferson LS, Vary TC. Altered expression of eukaryotic initiation factor 2B in skeletal muscle during sepsis. Am J Physiol Endocrinol Metab 1996;270: E43-E50.

[12] Shah OJ, Anthony JC, Kimball SR, Jefferson LS. 4E-BP1 and S6K1: translational integration sites for nutritional and hormonal information in muscle. Am J Physiol Endocrinol Metab 2000;279:E715-29.

[13] Duncan R, Hershey JWB. Initiation factor protein modifications and inhibition of protein synthesis. Mol Cell Biol 1987;7:1293-5.

[14] Karim MM, Hughes JMX, Warwicker J, Scheper GC, Proud CG, McCarthy JEG. A quantitative molecular model for modulation of mammalian translation by the eIF4E-binding protein 1. J Biol Chem 2001;276:20750-7.

[15] Lang CH, Frost RA, Jefferson LS, Kimball SR, Vary TC. Endotoxin-induced changes in muscle protein synthesis is associated with changes in eIF2B, eIF4E and IGF-I. Am J Physiol Endocrinol Metab 2000;278:E1133-43.

[16] Lang CH, Frost RA. Differential effect of sepsis on ability of leucine and IGF-I to stimulate skeletal muscle translation initiation. Am J Physiol Endocrinol Metab 2004;287:E721-30.

[17] Avruch J, Belham C, Weng Z, Hara K, Yonezawa K. The p70 S6 kinase integrates nutrient and growth signals to control translational capacity. Prog Mol Subcell Biol 2001;26:115-54.

[18] Dufner A, Thomas G. Ribosomal S6 kinase signaling and the control of translation. Exp Cell Res 1999;253:100-9.

[19] Pende M, Um SH, Mieulet V, Sticker M, Goss VL, Mestan J, et al. *S6K1*$^{-/-}$/*S6K2*$^{-/-}$ mice exhibit perinatal lethality and rapamycin-sensitive 5′ -terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase–dependent S6 kinase pathway. Mol Cell Biol 2004;24:3112-24.

[20] Stolovich M, Tang H, Hornstein E, Levy G, Cohen R, Bae SS, et al. Transduction of growth or mitogenic signals into translational activation of TOP mRNAs is fully reliant on the phosphatidylinositol 3-kinase-mediated pathway but requires neither S6K1 nor rpS6 phosphorylation. Mol Cell Biol 2002;22:8101-13.

[21] Lang CH, Silvis C, Deshpande N, Nystrom GJ, Frost RA. Endotoxin stimulates in vivo expression of inflammatory cytokines TNFα, IL-1β, IL-6 and HMBG1 in skeletal muscle. Shock 2003;19: 538-46.

[22] Frost RA, Nystrom GJ, Lang CH. LPS regulates the expression of proinflammatory cytokines in C2C12 myoblasts. Am J Physiol Regul Integr Comp Physiol 2002;283:R698-R709.

[23] Zamir O, Hasselgren PO, Kunkel SL, Frederick J, Higashiguchi T, Fischer JE. Evidence that tumor necrosis factor participates in the regulation of muscle proteolysis during sepsis. Arch Surg 1992;127: 170-4.

[24] Lang CH, Frost RA, Nairn AC, MacLean DA, Vary TC. TNF-α impairs heart and skeletal muscle protein synthesis by altering translation initiation. Am J Physiol Endocrinol Metab 2002;282: E336-47.

[25] Cooney R, Kimball SR, Eckman R, Maish G, Shumate M, Vary TC. TNF-binding protein ameliorates inhibition of skeletal muscle protein synthesis during sepsis. Am J Physiol Endocrinol Metab 1999;276: E611-7.

[26] Garcia-Martinez C, Lopez-Soriano FJ, Argiles JM. Acute treatment with tumour necrosis factor-alpha induces changes in protein metabolism in rat skeletal muscle. Mol Cell Biochem 1993; 125:11-8.

[27] Breuille D, Voisin L, Contrepois M, Arnal M, Rose F, Obled C. A sustained rat model for studying the long-last catabolic state of sepsis. Infect Immun 1999;67:1079-85.

[28] Espat NJ, Cenden JC, Bierle EA, Auffenberg TA, Rosenberg J, Russell DA, et al. PEG-BP-30 monotherapy attenuates the cytokine-mediated inflammatory cascade in baboon *Escherichia coli* septic shock. J Surg Res 1995;59:153-9.

[29] Kimball SR, Jurasinski CV, Lawrence JC, Jefferson LS. Insulin stimulates protein synthesis in skeletal muscle by enhancing the association of eIF-4E with eIF-4G. Am J Physiol Cell Physiol 1997; 272:C754-9.

[30] Martin-Perez J, Thomas G. Ordered phosphorylation of 40S ribosomal protein S6 after serum stimulation of quiescent 3T3 cells. Proc Natl Acad Sci U S A 1983;80:926-30.

[31] Jurasinski CV, Kilpatrick L, Vary TC. Amrinone prevents muscle protein wasting during chronic sepsis. Am J Physiol Endocrinol Metab 1995;268:E491-E500.

[32] Gingras A-C, Raught B, Sonenberg N. Regulation of translation initiation by FRAP/TOR. Genes Dev 2001;15:807-26.

[33] Peterson RT, Beal PA, Comb MJ, Schreiber SL. FKBP12-rapamycin–associated protein (FRAP) autophosphorylates at serine 2481 under translationally repressive conditions. J Biol Chem 2000;275: 7416-23.

[34] Holz MK, Blenis J. Identification of S6 kinase 1 as a novel mammalian target of rapamycin (mTOR)-phosphorylating kinase. J Biol Chem 2005;280:26089-93.

[35] Martin DE, Hall MN. The expanding TOR signaling network. Curr Opin Cell Biol 2005;17:158-66.

[36] Frost RA, Nystrom GJ, Lang CH. Tumor necrosis factor-α decreases insulin-like growth factor-I messenger ribonucleic acid expression in C2C12 myoblasts via a Jun N-terminal kinase pathway. Endocrinology 2003;144:1770-9.

[37] Fan J, Li YH, Bagby GJ, Lang CH. Modulation of inflammation-induced changes in insulin-like growth factor (IGF)-I and IGF binding protein-1 by anti-TNF antibody. Shock 1995;4:21-6.

[38] Yumet G, Shumate ML, Bryant P, Lang CH, Cooney RN. Tumor necrosis factor mediates hepatic growth hormone resistance during sepsis. Am J Physiol Endocrinol Metab 2002;283:E472-81.

[39] Charters Y, Grimble RF. Effect of recombinant human tumour necrosis factor α on protein synthesis in liver, skeletal muscle and skin of rats. Biochem J 1989;258:493-7.

[40] Kettelhut IS, Goldberg AL. Tumor necrosis factor can induce fever in rats without activating protein breakdown in muscle or lipolysis in adipose tissue. J Clin Invest 1988;81:1384-9.

[41] Flores EA, Bistrian BR, Pomposelli JJ, Dinarello CA, Blackburn GL, Istfan NW. Infusion of tumor necrosis factor/cachectin promotes muscle catabolism in the rat. A synergistic effect with interleukin 1. J Clin Invest 1989;83:1614-22.

[42] Cheema IR, Hermann C, Postell S, Holifield B. Effect of tumour necrosis factor-alpha on total myofibrillar and sarcoplasmic protein synthesis and polysomal aggregation in rat skeletal muscle. Cytobios 1999;97:133-9.

[43] Li YP, Reid MB. NF-κB mediates the protein loss induced by TNF-alpha in differentiated skeletal muscle myotubes. Am J Physiol Regul Integr Comp Physiol 2000;279:R1165-70.

[44] Frost RA, Lang CH, Gelato MC. Transient exposure of human myoblasts to TNFα inhibits serum and IGF-I stimulated protein synthesis. Endocrinology 1997;138:4153-9.

[45] Fong Y, Moldawer LL, Marano M, Wei H, Barber A, Manogue K, et al. Cachectin/TNF or IL-1α induces cachexia with redistribution of body proteins. Am J Physiol Regul Integr Comp Physiol 1989;256: R659-65.

[46] Alvarez B, Quinn LS, Busquets S, Lopez-Soriano FJ, Argiles JM. Direct effects of tumor necrosis factor alpha on murine skeletal muscle cell lines. Biomodal effects of protein metabolism. Eur Cytokine Netw 2001;12:399-410.

[47] Odedra BR, Bates PC, Millward DJ. Time course of the effect of catabolic doses of corticosterone on protein turnover in rat skeletal muscle. Biochem J 1983;214:617-27.

[48] Hall-Angeras M, Angeras U, Zamir O, Hasselgren PO, Fischer JE. Interaction between corticosterone and tumor necrosis factor stimulated protein breakdown in rat skeletal muscle, similar to sepsis. Surgery 1990;108:460-6.

[49] Li YH, Fan J, Lang CH. Role of glucocorticoids in mediating endotoxin-induced changes in IGF-I and IGF binding protein-1. Am J Physiol Regul Integr Comp Physiol 1997;272:R1970-7.

[50] Tiao G, Fagan J, Roegner V, Lieberman M, Wang JJ, Fischer JE, et al. Energy-ubiquitin–dependent muscle proteolysis during sepsis in rats is regulated by glucocorticoids. J Clin Invest 1996; 97:339-48.

[51] Saghir AN, Tuxworth WJ, Hagedorn CH, McDermott PJ. Modifications of eukaryotic initiation factor 4F (eIF4F) in adult cardiocytes by adenoviral gene transfer: differential effects on eIF4F activity and total protein synthesis rates. Biochem J 2001;356:557-66.

[52] Terada N, Patel HR, Takase K, Kohno K, Narin AC, Gelfand EW. Rapamycin selectively inhibits translation of mRNAs encoding elongation factors and ribosomal proteins. Proc Natl Acad Sci U S A 1994;91:1147-8.