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Low-energy laser irradiation enhances de novo protein synthesis via its effects on translation-regulatory proteins in skeletal muscle myoblasts

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Abstract

Low-energy laser irradiation (LELI) drives quiescent skeletal muscle satellite cells into the cell cycle and enhances their proliferation, thereby promoting skeletal muscle regeneration. Ongoing protein synthesis is a prerequisite for these processes. Here, we studied the signaling pathways involved in the LELI regulation of protein synthesis. High levels of labeled [^{35}S]methionine incorporation were detected in LELI cells as early as 20 min after irradiation, suggesting translation of pre-existing mRNAs. Induced levels of protein synthesis were detected up until 8 h after LELI implying a role for LELI in de novo protein synthesis. Elevated levels of cyclin D1, associated with augmented phosphorylation of the eukaryotic initiation factor 4E (eIF4E) and its inhibitory binding protein PHAS-I, suggested the involvement of LELI in the initiation steps of protein translation. In the presence of the MEK inhibitor, PD98059, eIF4E phosphorylation was abolished and levels of cyclin D1 were dramatically reduced. The LELI-induced PHAS-I phosphorylation was abolished after preincubation with the PI3K inhibitor, Wortmannin. Concomitantly, LELI enhanced Akt phosphorylation, which was attenuated in the presence of Wortmannin. Taken together, these results suggest that LELI induces protein translation via the PI3K/Akt and Ras/Raf/ERK pathways.

Keywords: Laser irradiation; Myoblast; Protein translation; PHAS-I; Akt; eIF4E

1. Introduction

Skeletal muscle regeneration is dependent on the proliferation and differentiation of satellite cells [1–4]. These cells are normally quiescent; however, in response to stimuli such as myotrauma, they become activated, proliferate and fuse into existing muscle fibers, or form new myofibers [5,6]. Recent studies have shown that low-energy laser irradiation (LELI) significantly enhanced the regeneration process; LELI of injured limb muscle in rat and toad induced young myofiber formation by two- and eightfold, respectively [7,8]. LELI has been shown to modulate other various biological processes such as increasing mitochondrial respiration and ATP synthesis and accelerating wound healing [9,10]. Recently, it was shown in ischemic heart that mitochondria were preserved and ATP-content was higher in irradiated hearts as compared to nonirradiated ones [11]. The most commonly used irradiation apparatus is the helium–neon (He–Ne) laser, which projects a beam at an optimal wavelength of 632.8 nm [12,13].

Extracts from crushed adult skeletal muscles or hepatocyte growth factor (HGF) supplementation, in vitro and in vivo, have been shown to induce cell-cycle entry of dormant satellite cells [14–16]. Likewise, we recently showed that LELI promotes activation of quiescent satellite cells, and enhances their proliferation and survival in both mass cultures and isolated muscle fibers [17,18]. LELI promotes cell proliferation by inducing tyrosine-kinase receptor phosphorylation, which in turn activates the extracellular signal-regulated protein kinase ERK1/2 [19], followed by the induction of early-G_1 phase regulatory proteins [17]. Previous studies have shown that induction of early-G_1 phase regulatory proteins, such as c-myc [20] and cyclin D1 [21], requires de novo mRNA and protein synthesis, resulting from translation of pre-existing mRNAs [22–25]. Many of these mRNAs typically have a highly structured 5′ -UTR,

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upstream AUGs and a poor sequence context for the initiating AUG [26–29]. Eukaryotic initiation factor 4E (eIF4E) is a major regulator of cap-dependent mRNA translation in response to proliferative stimuli such as hormones, growth factors and mitogens [30–34]. Three mechanisms are known to regulate eIF4E: (i) modulation of eIF4E expression levels [27,35]; (ii) phosphorylation of eIF4E at Ser^{209}, which increases its affinity to capped mRNA [36]; and (iii) phosphorylation-dependent dissociation of a translational-repressor protein from eIF4E (i.e. protein heat and acid stable; PHAS-I, also referred to as eIF4E binding protein-1 4EBP1; [37,38]). The non- or partially phosphorylated form of PHAS-I, which strongly interacts with eIF4E, limits the latter's availability of eIF4E to the translation process. The mammalian target of rapamycin (mTOR), a downstream kinase in the phosphoinositide 3-kinase (PI3K) pathway, is thought to phosphorylate PHAS-I [39]. The fully phosphorylated PHAS-I dissociates from eIF4E, allowing the latter to form the initiation complex and translation to proceed [29,38,40].

Previously, it was shown that irradiation with He–Ne laser increased cytosolic and mitochondrial protein synthesis in isolated rat hepatocytes [41]. The present study was aimed at investigating the effect of LELI on de novo protein synthesis in the muscle cell system and to define the involved signaling pathways. We focused on the regulation of translation initiation, which is the rate-limiting step in protein synthesis [30].

2. Materials and methods

2.1. Cell culture

i28 mouse myogenic cells [17,42] were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 20% fetal calf serum (FCS). For the experiments, cells were plated at 7×10^4 cells/60-mm petri dish, and maintained in serum-free DMEM for 36 h.

2.2. Laser irradiation

After 36 h of FCS deprivation, cells were irradiated through a grid composed of 1.8×1.8 mm squares, for 3 s per square, with a He–Ne laser (632.8 nm, 4.5 mW; 1.8-mm beam diameter; Ealing Electro-Optics, Holliston, MA). This methodology equalizes the effect on cells in the entire tissue-culture plate [17,19]. After irradiation, cells were maintained in serum-free DMEM for the indicated time periods. Control, nonirradiated cells were maintained under the same conditions as the LELI cells.

2.3. Metabolic labeling

Cells were deprived of methionine for 30 min in methionine-free DMEM. [^{35}S]methionine (1 μCi/ml, specific activity 1175 Ci/mmol; New England Nuclear, Boston, MA) was added for 2 h to enable labeling of de novo protein synthesis. Labeled cells were lysed in ice-cold NP-40-containing lysis buffer, sonicated and clarified by centrifugation at $10{,}000 \times g$. Aliquots of 10 μl of whole-cell extracts were dripped onto glass microfiber filters (Schleicher and Schuell, Dassel, Germany), and proteins were precipitated in ice-cold 10% (v/v) trichloroacetic acid (TCA) followed by 5% TCA. Filters were dried for 30 min and then immersed in Ultima Gold scintillation fluid (Packard, Downers Grove, IL). Radioactivity was counted in a Tri-Carb 1600CA scintillation counter (Packard).

2.4. Immunoprecipitation of eIF4E

Cells were lysed in NP-40-containing immunoprecipitation lysis buffer as previously described [19]. Protein extracts were sonicated and normalized for protein content (BCA kit, Pierce, Rockford, IL). The lysates (50-μg protein) were incubated overnight at 4 °C with anti-eIF4E monoclonal antibody (Transduction Laboratories, Lexington, KY) diluted 1:3000. This was followed by incubation with protein A–Sepharose beads (Amersham Pharmacia Biotech, Uppsala, Sweden) mixed at a ratio of 1:2 with protein L beads (CBD, Rehovot, Israel) for 2 h at RT. The resulting immunocomplex was washed three times in PBS and subjected to electrophoresis (SDS-PAGE).

2.5. Western blot analysis

Cells were lysed in NP-40-based lysis buffer as previously described [17]. Extracts were sonicated with an ultrasonic cell disrupter (Microson, Farmingdale, NY) and clarified by centrifugation at $10{,}000 \times g$. Proteins were subjected to SDS-PAGE and transferred to nitrocellulose filters (Schleicher and Schuell). Filters were blocked with TBS-T (50 mM Tris–HCl, pH 7.5, 150 mM NaCl and 0.5% Tween 20) containing 5% (w/v) BSA, incubated overnight at 4 °C with the primary antibodies, washed with TBS-T and incubated for 1 h with HRP-conjugated goat anti-mouse or goat anti-rabbit IgG (Zymed laboratories, San Francisco, CA). Signals were generated with an ECL kit (Pierce). Densitometric analyses of the signals were performed using the Gel-Pro software.

The following primary antibodies were used: anti-PHAS-I polyclonal antibody [43], diluted 1:3000; anti-phosphorylated ERK polyclonal antibody (Promega, Madison, WI), diluted 1:5000 and 1:2000, respectively; anti-phosphoserine polyclonal antibody (Chemicon, Temecula, CA), diluted 1:1500; anti total Akt (1:1000, Cell Signaling, Beverly, MA); anti-phosphorylated Akt monoclonal antibody (Promega), diluted 1:2500; anti-eIF4E monoclonal antibody (Transduction Laboratories), diluted 1:3000; monoclonal anti-cyclin D1 antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA); and monoclonal anti α-tubulin (1:1000, Oncogene, Darmstadt, Germany).

2.6. Statistics

Unless otherwise indicated, data were analyzed using one-way or two-way ANOVA. Comparisons of individual groups were performed using Tuckey honest test. The significance level was set to $P<0.05$ for all experiments.

3. Results

3.1. LELI promotes de novo protein synthesis in serum-starved myogenic cells

The effect of LELI on de novo protein synthesis was tested in i28 myoblasts that had been rendered quiescent by serum deprivation for 36 h. Cells were labeled for 2 h with [^{35}S]methionine immediately after irradiation or 2, 4 or 6 h thereafter. Control, nonirradiated cells underwent the same experimental procedure. The overall level of protein synthesis was significantly higher in the LELI groups compared to controls (two-way ANOVA $F(1,8)=18.41$; $P<0.0026$; Fig. 1A). Specifically, LELI induced threefold the incorporation of [^{35}S]methionine over the control during the first 2 h after irradiation. In both LELI and control cells, methionine incorporation was significantly reduced during the second 2-h period, remaining relatively low in controls but induced again in the LELI cells. A fourfold difference in [^{35}S]methionine incorporation was observed between control and LELI-treated cells at 8 h post-irradiation.

To study the effect of LELI on protein translation process, cells were labeled with [^{35}S]methionine for a short interval of 20 min immediately after LELI (Fig. 1B). During that time period, a significant increase in [^{35}S]methionine incorporation was observed in the LELI vs. control cells (*t*-test: two-sample assuming unequal variances, $P=0.012$), suggesting the induction of protein translation of pre-existing mRNA upon LELI.

To confirm this hypothesis, we tested the expression of cyclin D1, which is subjected to translational control [35,44–47]. Higher cyclin D1 levels (approximately fourfold) were detected in the LELI-treated cells than in their respective controls as early as 6 min post-irradiation. The levels of cyclin D1 were sustained at 15 min post-irradiation, however, were lower than those in the proliferating myoblasts (Fig. 1C).

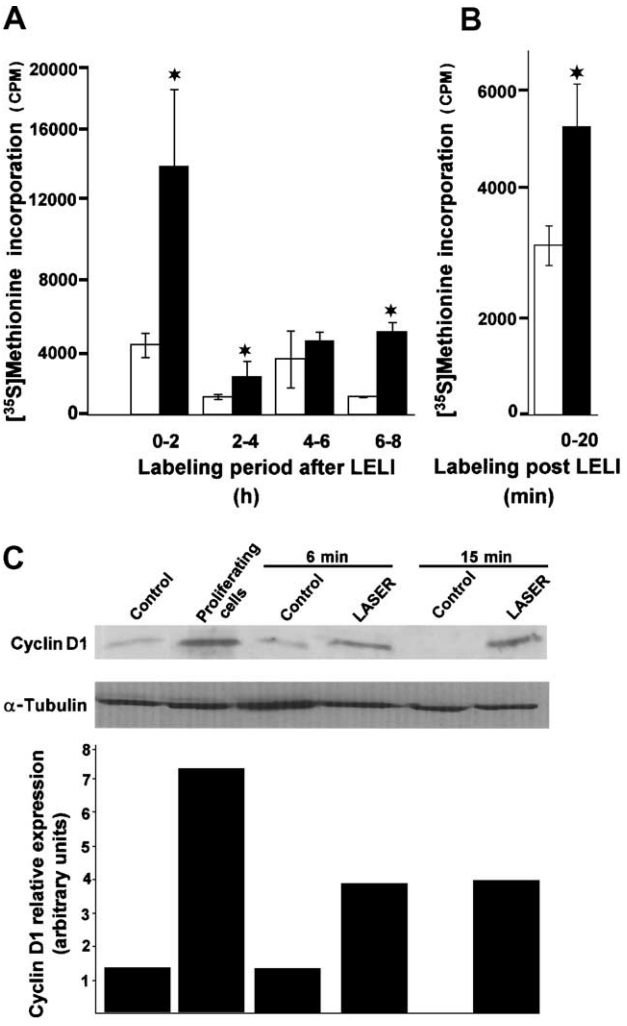

Fig. 1. Time-course analysis of the effect of LELI on total protein synthesis. i28 cells were serum-deprived for 36 h, after which they were either laser-irradiated for 3 s (solid columns) or served as controls (open columns). After LELI, cells were incubated for the indicated periods and metabolically labeled. [^{35}S]methionine was added for the last 2 h of the incubation (A), or for 20 min immediately after irradiation (B). Incorporation of labeled methionine into proteins was determined in 10-μl aliquots of TCA-precipitated whole-cell extract. These data are the mean ± S.E. of three independent experiments. Asterisks indicate a significant difference ($P<0.05$) from respective controls. (C) Cyclin D1 protein expression was visualized at various times after irradiation using Western blot analysis (upper panel). Densitometric analysis of cyclin D1 expression levels relative to α-tubulin (lower panel). Proliferating cells were those maintained in 20% FCS-containing medium.

3.2. LELI induces eIF4E phosphorylation in myoblasts

eIF4E has been shown to play a key role in protein translation. To study the effect of LELI on the expression and phosphorylation of eIF4E, serum-deprived i28 cells were allocated to one of three experimental groups: (i) nonirradiated control cells (control); (ii) LELI-treated cells (LASER); (iii) 10% FCS-supplemented cells, serving as a positive control for stimulation of proliferation. These cells were serum-refed for the time needed to irradiate one petri dish, and thereafter were maintained in DMEM alone (10% FCS). Cells were harvested at 15 and 90 min post-irradiation and analyzed for the expression or phosphorylation of eIF4E (Fig. 2). eIF4E phosphorylation was induced after 15 min in LELI- and FCS-treated cells. In the LELI cells, high levels of phosphorylated eIF4E were maintained until 90 min after irradiation (Fig. 2A), and were compatible to those in the proliferating cells. Total levels of eIF4E were not significantly affected by either LELI or serum re-feeding (Fig.

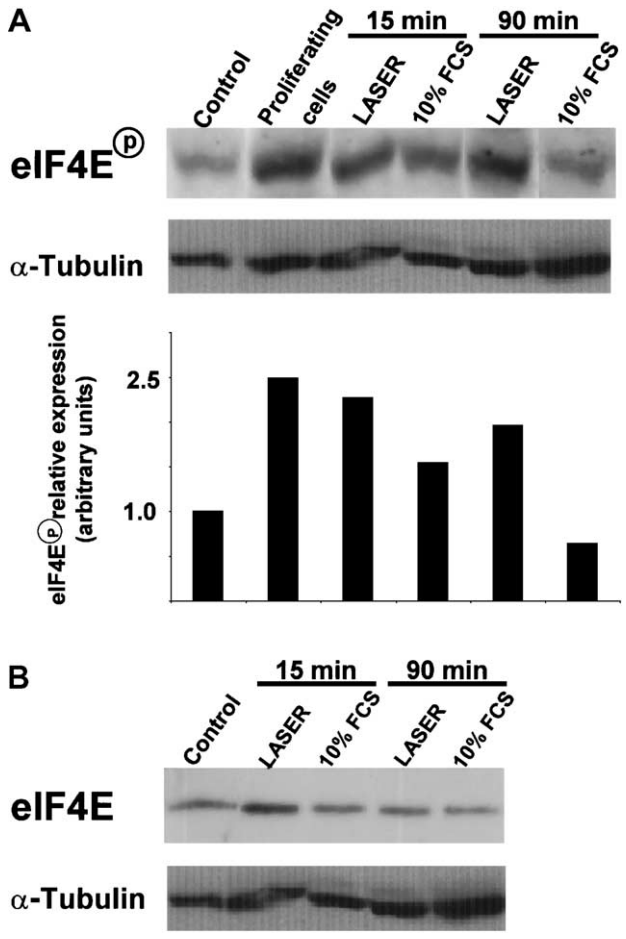

Fig. 2. LELI induces phosphorylation of eIF4E but does not affect its expression levels. i28 cells were serum-deprived for 36 h, after which they were either irradiated for 3 s (LASER) or refed with DMEM containing 10% FCS (10% FCS) for the time needed to laser irradiate one petri dish. Upon termination of irradiation the FCS-containing medium was replaced with DMEM. Cells were harvested at the times indicated above the lanes and equal amounts of protein (50 μg) were electrophoresed and blotted. Blots were probed with antibodies against phospho-eIF4E (A) and total eIF4E (B). Equal loading of proteins was verified by reprobing the blots with an antibody against α-tubulin. Bands identifying the relative expression of phosphorylated eIF4E were quantified by densitometric analysis and normalized to that of α-tubulin (A, lower panel). Results represent one of four independent experiments.

2B), suggesting that this protein is not regulated at the translational level.

3.3. LELI regulates eIF4E via the MAPK/ERK pathway

eIF4E is rapidly phosphorylated on Ser^{209} in response to mitogens and growth stimuli via the MAPK/ERK pathway [48–50]. The MAPK/ERK-mediated effect of LELI on eIF4E phosphorylation was tested in the presence or absence of the noncompetitive MEK inhibitor, PD098059 [51]. Serum-deprived i28 cells were supplemented with DMEM containing 50 μM PD098059, 30 min prior to irradiation. Positive-control plates were supplemented with 10% FCS containing 50 μM PD098059. Both LELI and FCS substantially induced phosphorylation of eIF4E in the absence of PD098059 (Fig. 3, lane 1 vs. lanes 2 and 4, Fig. 2A). This phosphorylation was significantly reduced after incubation with PD098059 (Fig. 3, lanes 3 and 5). Activation of the MAPK pathway is essential for cyclin D1 expression and provides a link between mitogenic signaling and cell-cycle progression [52]. Indeed, elevated levels of cyclin D1 were observed in the LELI and serum-refed compared to control cells (Fig. 3, lanes 1, 2 and 4; Fig. 1C). These levels were

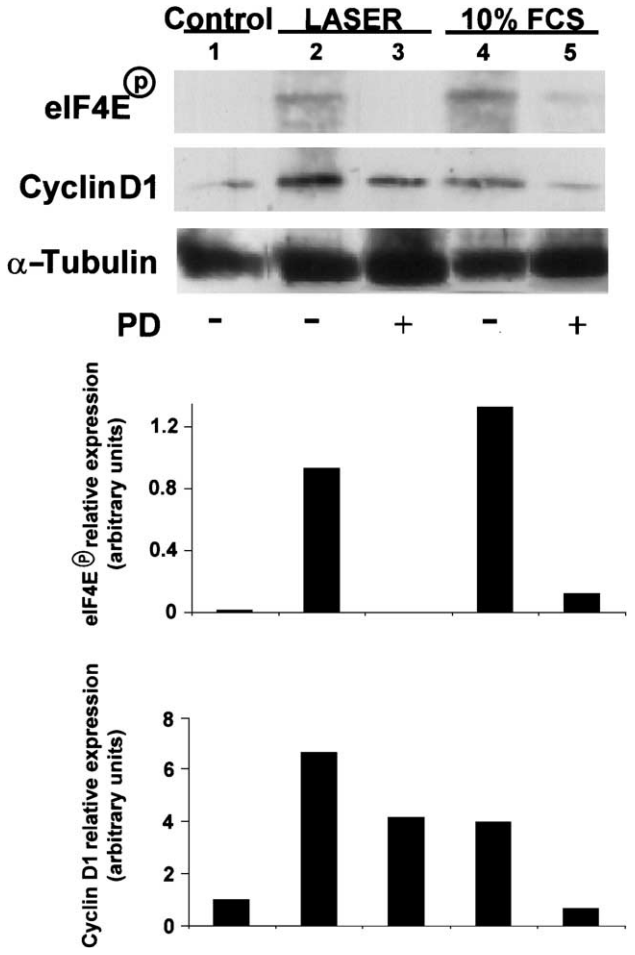

Fig. 3. PD098059 inhibits LELI-induced eIF4E activation. i28 cells were serum-deprived for 36 h with DMEM, followed by preincubation with 50 μM PD098059 (PD) for 30 min (+). Cells that were not preincubated with the inhibitor are marked with (−). Cells were then irradiated (LASER) or supplemented with DMEM–10% FCS (10% FCS), and harvested 15 min after irradiation or refeeding. Upper panel: Equal amounts (60 μg) of protein were either immunoprecipitated with anti-eIF4E antibody or reacted with anti-cyclin D1 antibody. The immunoprecipitates were analyzed on immunoblots using an anti-phosphoserine antibody. Equal protein loading per lane was confirmed by immunoblot analysis with α-tubulin antibody. Lower panel: Bands identifying the relative expression of phosphorylated eIF4E and cyclin D were quantified by densitometric analysis and normalized to that of α-tubulin. Data represent two independent experiments.

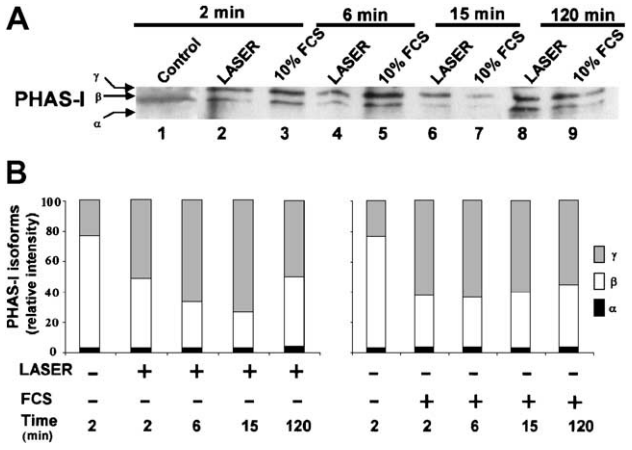

Fig. 4. LELI-induced PHAS-I phosphorylation. (A) i28 cells were treated as in Fig. 2, lysed 30 min after irradiation, and 60-μg protein was analyzed by immunoblotting with anti-PHAS-I antibody. (B) Signals of the individual PHAS-I forms were analyzed by densitometry and expressed as percentages of the total intensity of the three PHAS-I forms. These data are representative of three separate experiments.

markedly reduced in the presence of PD098059 (Fig. 3, lanes 3 and 5). Taken together, our results indicate that LELI-induced eIF4E phosphorylation is mediated via the MAPK/ERK pathway.

3.4. LELI affects PHAS-I phosphorylation

PHAS-I is an eIF4E-binding protein that mediates cap-dependent translation [27]. In the presence of mitogens, PHAS-I is phosphorylated and dissociates from eIF4E [53,54]. Three isoforms of PHAS-I were detected in myoblasts (Fig. 4A): the non- and partially phosphorylated isoforms that bind eIF4E (α and β), and the fully phosphorylated, slowest migrating isoform (γ), which does not bind eIF4E [37,38]. In serum-deprived cells, the dominant β isoform of PHAS-I represented 80% of the total protein (Fig. 4A, lane 1 and Fig. 4B). LELI and serum-refeeding induced rapid phosphorylation of PHAS-I as early as 2 min after treatment (Fig. 4A, lane 1 vs. lanes 2 and 3, and Fig. 4B). High levels of PHAS-I phosphorylation were maintained until 15 min after irradiation or serum-refeeding (Fig. 4A, lane 1 vs. lanes 4,5 and 6,7 and Fig. 4B). At 120 min post-irradiation, the ratio between β and γ isoforms was equalized in the LELI-treated cells (Fig. 4A, lane 8). In the FCS-refed cells, the high ratio between the γ and β isoforms remained unchanged during consequent periods of time, up to 120 min after refeeding (Fig. 4A, lane 9 and Fig. 4B).

3.5. LELI affects PHAS-I phosphorylation in a PI3K-dependent manner

PI3K mediates growth factor and hormone-dependent phosphorylation of PHAS-I [33,55,56]. Its involvement in LELI-induced PHAS-I phosphorylation was examined, using the specific PI3K inhibitor Wortmannin. Serum-deprived myoblasts were LELI-treated, harvested 30 min post-irradiation and immunoblotted to analyze PHAS-I phosphorylation.

Laser irradiation resulted in significant PHAS-I phosphorylation, evidenced by high levels of the fully phosphorylated γ isoform (Fig. 5, lane 1 vs. lane 2). Some effect of Wortmannin on PHAS-I phosphorylation could be seen at the concentrations of 10^{-9} to 10^{-7} M (Fig. 5, lanes 3 to 5). At higher concentration of 10^{-6} M Wortmannin [57] there was a threefold reduction in the level of the γ isoform obtained, reversing the γ/β ratio approximately to control levels (Fig. 5, lane 1 vs. lane 6). These data suggest that LELI regulates PHAS-I phosphorylation in a PI3K-dependent pathway.

We next examined the effect of LELI on Akt phosphorylation, a downstream target of PI3K [58]. Serum-deprived i28 cells were irradiated or serum-refed and lysed 6, 15 and 30 min later. Whole-cell extracts were analyzed by SDS-PAGE and reacted with an antibody to the phosphorylated form of Akt. Both LELI and FCS induced Akt phosphorylation relative to controls (Fig. 6A). A densitometric analysis revealed that the LELI effect was lower compared to that of FCS, however, less transient (Fig. 6A, lower panel). Neither LELI nor FCS treatments affected total Akt expression levels (Fig. 6B).

Wortmannin supplemented at the concentrations of 10^{-8} and 10^{-7} M did not affect the level of phosphorylated Akt (Fig. 6C). Akt phosphorylation was suppressed at a higher

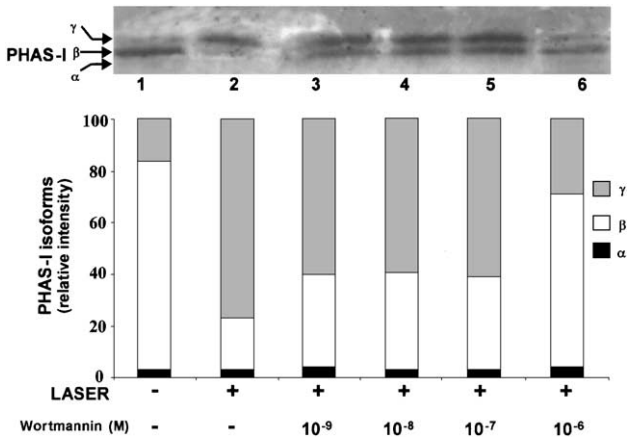

Fig. 5. LELI-induced PHAS-I phosphorylation is Wortmannin-sensitive. i28 cells were serum-deprived for 36 h, after which they were preincubated for 30 min with various concentrations of Wortmannin, after which they were or were not irradiated. Equal amounts of proteins were analyzed by 17% SDS-PAGE, transferred to a nitrocellulose membrane, and immunoblotted with an antibody against PHAS-I. A representative autoradiograph of two experiments is shown (upper panel). Signals of the individual PHAS-I forms were quantitated by densitometry and expressed as percentages of the total intensity of the three PHAS-I forms (lower panel).

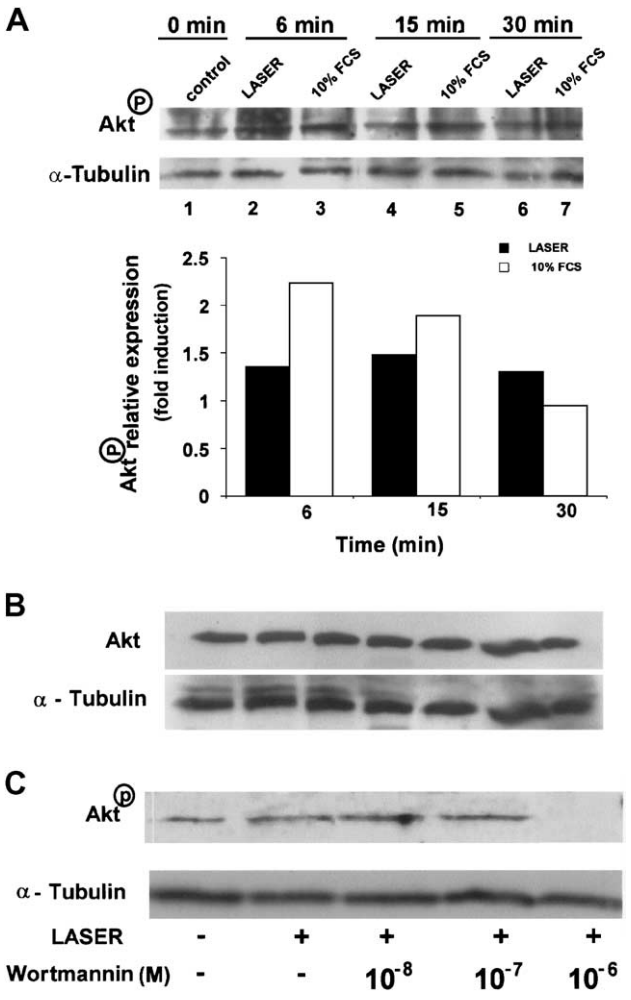

Fig. 6. LELI-induced Akt phosphorylation is Wortmannin-sensitive. i28 cells were treated as described in Fig. 2. Cells were then lysed at the indicated periods of time after irradiation. Equal amounts of proteins were analyzed by 12.5% SDS-PAGE, transferred to a nitrocellulose membrane, and immunoblotted with an antibody against phosphorylated (Ser^{473}) Akt or α-tubulin (A). A representative autoradiograph of two experiments is shown (A, upper panel). Signals of Akt were quantitated by densitometry, normalized to the level of α-tubulin and are presented relative to the amount of phosphorylated Akt detected zero time (control) (A, lower panel). A parallel experiment to (A) shows that total Akt expression was not affected by LELI nor by serum treatment (B). (C) i28 cells were treated for 30 min with various concentrations of Wortmannin and then either irradiated (+) or kept aside (−).

level of 10^{-6} M, arguing for activation of the PI3K pathway following LELI.

4. Discussion

In light of previous studies demonstrating enhanced satellite cell proliferation and cell-cycle progression following LELI, the working hypothesis of this study was that these processes are mediated by increased de novo protein synthesis. Indeed, a rapid and significant induction of de novo protein synthesis in skeletal muscle satellite cells was evident within the first 20 min after LELI. This finding is in agreement with an earlier study showing a higher protein synthesis with a similar time course, in He–Ne laser-irradiated rat hepatocytes [41]. The augmentation in newly synthesized proteins implies that shortly after irradiation, translation of pre-existing mRNAs has occurred. Most of these pre-existing mRNAs possess complex 5′ secondary structures and code for growth-related genes, such as ornithine decarboxylase (ODC; [59,60]) and cyclin D1 [45,47,61]. The translation of such mRNAs is selectively enhanced upon mitogenic stimulation [35]. Indeed, induction of cyclin D1 expression was detected in LELI cells as early as 6 min after irradiation, suggesting that the increased synthesis following LELI involves the translation of proteins that are required for entrance and progression through the G_1 phase of the cell cycle.

Induction of protein synthesis during the first 2 h after irradiation was followed by a decline and a second induction 6 to 8 h after irradiation in LELI cells. These fluctuations in protein synthesis resemble those seen under mitogenic stimulation, and may result from the effect of LELI on the synthesis of proteins participating in later stages of G1 phase of the cells cycle [62].

To elucidate the contribution of LELI to the regulatory pathways of protein synthesis, we studied its effect on the levels and activity of eIF4E, which plays a key role in translation initiation. LELI induced eIF4E phosphorylation without affecting its expression levels. The MEK inhibitor PD098059 abolished eIF4E phosphorylation, suggesting that LELI-induced eIF4E phosphorylation is Ras/Raf/ERK-dependent. This finding confirms our previous observations that LELI specifically activates the MAPK/ERK pathway and consequently induces satellite cell proliferation [17,19].

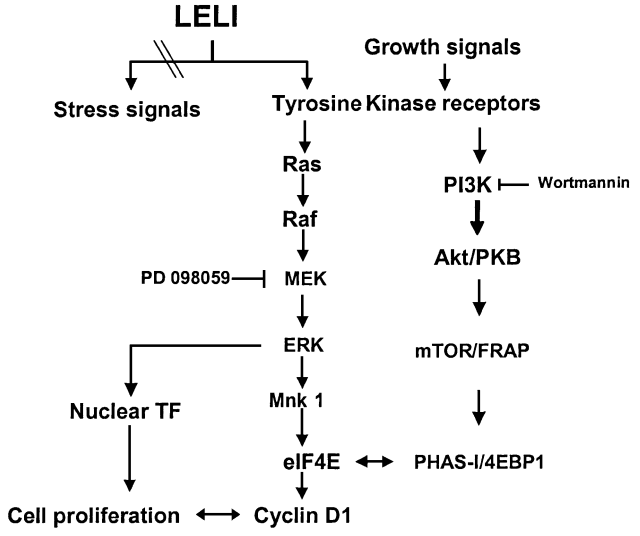

Fig. 7. A proposed model for LELI signaling in skeletal muscle satellite cells.

Although eIF4E phosphorylation is Ras/Raf/ERK-dependent, MAPK/ERK does not directly activate eIF4E. Previous studies have shown that p38 MAPK or MAPK/ERK phosphorylate (in vivo and in vitro) mitogen-activated protein kinase signal-integrating kinases 1 and 2 (Mnk1 and Mnk2), which in turn phosphorylate eIF4E [50,63–67]. In response to stress agents (NaCl or anisomycin), Mnk1 is phosphorylated by p38 MAPK but not by MAPK/ERK. Nonetheless, in the presence of growth stimuli, ERK was more potent at phosphorylating Mnk1 than p38 was [50]. Moreover, in contrast to Mnk1, Mnk2 retains significant activity in the presence of both ERK and p38 inhibitors (PD098059 and SB203580, respectively; [67]). Given that eIF4E phosphorylation was abolished in the presence of PD098059, and that LELI does not affect p38 MAPK phosphorylation [19], we suggest that LELI induces eIF4E phosphorylation via Mnk1 phosphorylation (Fig. 7).

eIF4E activity is also regulated by PI3K-dependent PHAS-I phosphorylation [39]. The fully phosphorylated isoform of PHAS-I dissociates from eIF4E, allowing initiation of translation [37,38]. In this study, we demonstrate that LELI induced the phosphorylation of PHAS-I, which was abolished by the addition of the PI3K inhibitor Wortmannin, suggesting this phosphorylation to be PI3K-dependent. Moreover, LELI induced the phosphorylation of Akt, a downstream target of PI3K in a Wortmannin-dependent manner (Fig. 7). It has been reported that Akt affects downstream survival and death factors as well as regulatory proteins involved in the control of translation [68]. Akt directly phosphorylates mTOR, which in turn induces PHAS-I phosphorylation [27,58,69,70]. Taken together, we suggest that PI3K-dependent phosphorylation of Akt mediates the effect of LELI on PHAS-I phosphorylation and eIF4E availability to the translation machinery.

In this study we demonstrated a prolonged effect of LELI on PHAS-I as well as on Akt phosphorylation compared to the FCS refed cells. This phenomenon might explain the prolonged effect observed for eIF4E phosphorylation, which occurs downstream to both Akt and PHAS-I. Such a prolonged effect of LELI on infracted heart was reported in vivo in previous studies [71]. Notably, FCS contains a mixture of various growth factors, which may account for the higher induction of phosphorylation.

We report here that LELI induces de novo protein synthesis by modulating the activity of key enzymes that regulate capped mRNAs translation. Consistent with the data presented in this and previous studies [17,19], we propose a model that summarizes our findings (Fig. 7). Identifying the consequences of LELI on translation initiation, which is a prerequisite for muscle proliferation and hypertrophy, is important for establishing the therapeutic potential of LELI in cases of muscle injury or atrophy.

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