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# Phosphoinositide 3-kinase regulates myogenin expression at both the transcriptional and post-transcriptional level during myogenesis

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It is well-established that phosphoinositide 3-kinase (PI3-kinase) regulates myogenesis by inducing transcription of myogenin, a key muscle regulatory factor, at the initiation of myoblast differentiation. In this study, we investigated the role of PI3-kinase in cells that have committed to differentiation. PI3-kinase activity increases during myogenesis, and this increase is sustained during the myogenic process; however, its function after the induction of differentiation has not been investigated. We show that LY294002, a PI3-kinase inhibitor, blocked myoblast fusion even after myogenin expression initially increased. In contrast to the inhibitory effects of LY294002 on myogenin mRNA levels during the initiation of differentiation, LY294002 blocked the accumulation of myogenin protein without affecting its mRNA level after differentiation was induced. Treatment with cycloheximide, a translation inhibitor, or actinomycin D, a transcription inhibitor, indicated that the stability of myogenin protein is lower than that of its mRNA. LY294002 inhibited the activities of several important translation factors, including eukaryotic elongation factor-2 (eEF2), by altering their phosphorylation status. In addition, LY294002 blocked the incorporation of [$^{35}$S]methionine into newly synthesized proteins. Since myogenin has a relatively short half-life, LY294002-mediated inhibition of post-transcriptional processes resulted in a rapid depletion of myogenin protein. In summary, these results suggest that PI3-kinase plays an important role in regulating the expression of myogenin through post-transcriptional mechanisms after differentiation has been induced.

**Keywords:** myogenesis; myogenin; PI3-kinase; half-life; eukaryotic elongation factor-2; translation factors

## Introduction

The differentiation of skeletal muscle cells is characterized by withdrawal from the cell cycle, alignment and elongation, activation of genes expressing muscle-specific proteins, and fusion of mononucleated myoblasts to form multinucleated myotubes (Molkentin and Olson 1996). Myoblast differentiation depends on the activities of muscle regulatory factors (MRFs) consisting of four members: MyoD, myogenin, Myf5, and MRF4, which contain identical basic helix-loop-helix (bHLH) regions (Brand-Saberi and Christ 1999). All of these factors can induce the differentiation of non-muscle cells into a muscle cell phenotype (Emerson 1990; Olson 1990; Weintraub et al. 1991). However, they function very differently during myogenesis. Myf5 and MyoD mediate the early events of myogenesis, presumably during the myogenic determination phase, whereas myogenin and MRF4 function later to cause terminal differentiation (Perry and Rudnick 2000). While MyoD is expressed in both undifferentiated and differentiated myoblasts, myogenin expression begins upon induction of differentiation and its mRNA level is sustained throughout myogenesis (Li et al. 1994). The importance of myogenin in myogenesis is based on the strict correlation of myogenin expression and the onset of differentiation in skeletal muscle cell lines (Edmondson and Olson 1989; Wright et al. 1989; Lassar and Musterberg 1994). In addition, homozygous inactivation of the myogenin gene causes accumulation of myoblasts that are arrested in their terminal differentiation program (Arnold and Braun 1996).

It is believed that phosphoinositide 3-kinase (PI3-kinase) is indispensable for myoblast differentiation. PI3-kinase inhibitors, such as LY294002 and wortmannin, or the dominant negative mutant form of p85α, the regulatory subunit of PI3-kinase, block myoblast fusion and the expression of muscle-specific proteins (Kaliman et al. 1996; Jiang et al. 1998). We have previously shown that PI3-kinase is involved in withdrawal from the cell cycle at the early stages of L6 myoblast differentiation (Woo and Kim 2006). Regulation of myogenesis by PI3-kinase is closely related to myogenin expression. There have been many reports demonstrating that PI3-kinase induces myogenin expression at the transcriptional level upon the initiation of differentiation (Kaliman et al. 1996; Jiang et al. 1998; Sarker and Lee 2004).

Although the importance of PI3-kinase activity has been demonstrated during the early stages of

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myogenesis, the role of PI3-kinase in later differentiation stages, i.e., after cells have committed to differentiation, has not been investigated. In this study, we examined the role of PI3-kinase after the first observed increase in myogenin expression. Since myogenin expression is sustained throughout myogenesis, we hypothesized that PI3-kinase may be involved in the maintenance of high myogenin protein levels, in addition to its established role in the initial induction of myogenin expression. Here, we demonstrate that PI3-kinase regulates myogenin expression not only at the transcriptional level during the early stages of myogenesis but also at the post-transcriptional level after cells have committed to differentiation. This contributes to the maintenance of high myogenin protein levels throughout myogenesis.

## Materials and methods

### *Cell culture*

L6 rat skeletal myoblasts (American Type Culture Collection, USA) were cultured for 3 days in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Differentiation was induced by changing the media to DMEM containing 5% horse serum (referred to as differentiation medium). Cell fusion was assessed as previously reported (Jeon et al. 1994). The cells were only considered to be fused if there was clear cytoplasmic continuity and the presence of at least three nuclei within the myotubes.

### *Western blotting*

Western blotting analysis was performed as previously described (Woo and Kim 2006). The eukaryotic elongation factor 2 (eEF2) antibody was prepared as previously described (Jeon et al. 1994). The antibodies for myogenin, β-actin and glyceraldehyde 3-phosphate (GAPDH) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibodies for phospho-p70 ribosomal protein S6 kinase ($p70^{S6K}$) on Thr389, phospho-ribosomal protein S6 (S6) on Ser235/236, phospho-eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) on Ser65, phospho-eEF2 on Thr56, eEF2 kinase (eEF2K) and phospho-eEF2K on Ser366 were purchased from Cell Signaling Technology (Beverly, USA). The proteins were detected using enhanced chemiluminescence (Amersham Bioscience, UK).

### *PI3-kinase activity assay*

PI3-kinase activity was determined as previously described (Woo et al. 2006). Briefly, the cell lysates (500 μg proteins) were incubated with an anti-p85α antibody (Cell Signaling Technology), and the immune-precipitates were incubated with 2.5 μg of phosphatidylinositol (PI) and 5 μCi of [γ-$^{32}$P]ATP (Amersham Bioscience) for 10 min at room temperature. The phospholipids were separated using thin-layer chromatography (TLC), and the radio-labeled phosphatidylinositol-3-phospate (PIP3) products were identified as previously described (Giorgino et al. 1997).

### *Pulse metabolic labeling*

Myoblasts were cultured in differentiation medium for 24 h with the indicated concentrations of LY294002 (Sigma, USA). The cells were washed with methionine-free DMEM and labeled with 20 μCi/ml of L-[$^{35}$S] methionine ([$^{35}$S]-Met) (Amersham Biosciences) for 1 h. After all the unincorporated [$^{35}$S]Met was removed by washing, the cells were harvested and lysates were prepared. Equal amounts of proteins were separated using 7–14% SDS-PAGE and stained with Coomassie R250. The incorporated radioactivity was detected by exposing the dried gel to X-ray film. The band density was quantified using the Scion Image program (Scion Corporation, USA).

### *RT-PCR*

Total RNA was isolated from cells using the RNAgents total RNA isolation system (Promega, UK), according to the manufacturer's instructions. The qRT-PCR was performed using the one-step premix RT-PCR system (iNtRON, Korea), according to the manufacturer's instructions. Briefly, RT-PCR reactions were prepared using a master RT-PCR mixture containing 2 μg of total RNA, 10 units of RNase inhibitor (New England Biolabs, Beverly, MA, USA) and specific primers for myogenin (5′-GCAGTGCCATCCAGTACATTGA GC-3′ and 5′-GGAAGGTGACAG ACATATCCTC-CAC-3′), or GAPDH (5′-ACCACAGTCCATGCCAT-CAC-3′ and 5′-TCC ACCACCCTGTTGCTGTA-3′). The reverse-transcribed cDNA templates were amplified using 25 cycles of 94°5 for 30 s, 59°C for 45 s, and 72°C for 30 s for myogenin, and 20 cycles of 94°C for 30 s, 50°C for 45 s, and 72°C for 40 s for GAPDH under non-saturation PCR amplification conditions. The RT-PCR products were separated on a 2% agarose gel and detected by staining with ethidium bromide.

### *Statistical analysis*

All the averaged data were expressed as mean ± SEM. The significance of the differences from the respective controls for each experiment was assayed using Student's *t*-test.

## Results

### *Sustained PI3-kinase activity is required for myoblast differentiation*

Once myoblasts are transferred into differentiation medium, they immediately commit to differentiation, which is associated with cell fusion and increased expression of muscle-specific proteins. The L6 myoblasts became multinucleated myotubes with spontaneous cell fusion in normal differentiation medium and the fusion index increased to greater than 60% after 72 h of culture under these conditions (Figure 1A). In this study, we examined PI3-kinase activity after the myoblasts had committed to differentiation. Cells were exposed to LY294002 at 24 h after inducing differentiation, which was confirmed by the onset of cell fusion and when approximately 10% of the nuclei were included in myotubes. In contrast to myoblasts cultured under normal conditions, which had a fusion index greater than 60%, the fusion index for the LY294002-treated cells remained at approximately 10% (Figure 1A). Therefore, there was no further cell fusion after LY294002 treatment, irrespective of the exposure time.

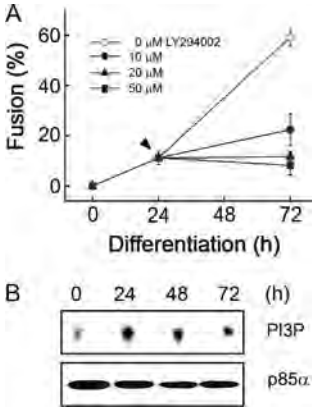


Figure 1. Sustained PI3-kinase activity is required for myoblast differentiation. (A) Myoblasts cultured in the mitogen-rich medium were induced to differentiate by transferring them into differentiation medium. At 24 h after inducing differentiation (arrowhead), the myoblasts were exposed to the indicated concentrations of LY294002 and cultured for a further 48 h. The cells were observed under a microscope, and the fusion index in 10 randomly chosen fields was measured, as described in the 'Materials and methods'. The values represent the average ± SEM for three independent experiments. (B) At the indicated culture times after inducing differentiation, the cells were harvested, and p85α was immunoprecipitated using an anti-p85α antibody. The PI3-kinase activity was assayed by separating the $^{32}$P-labeled PI3P, which was generated by PI3-kinase. The p85α protein represents the input for each sample. Results are representative of four independent experiments.

We then investigated the PI3-kinase activity by using the immunoprecipitated p85α, the regulatory subunit of PI3-kinase, from the myoblast lysates. The activity of PI3-kinase can be assayed by measuring the amount of exogenous PI phosphorylation into PI3P. The PI3-kinase activity was detected before inducing differentiation (0 h), but it further increased during the differentiation process (Figure 1B). The level at 24 h was approximately three times greater than that observed at 0 h (Figure 1B). The activity reached its peak at approximately 24–48 h after inducing differentiation, and this increase was relatively sustained, as there was only a slight reduction in activity detected. However, this increased activity is not likely to be due to increased levels of the enzyme, as there was no correlative increase in the amount of input, i.e., the levels of p85α did not change. These results suggest that PI3-kinase activity is required not only for the induction of differentiation but also after its onset.

Since PI3-kinase is involved in the regulation of myogenin expression (Kaliman et al. 1996), and myogenin is a key regulatory factor of myogenesis during the early stages of differentiation, the activity of PI3-kinase has been primarily studied in the context of differentiation initiation. Under normal culture conditions, the expression of myogenin was detected at 24 h following the induction of differentiation (Figure 2A), which is when cells had committed to this fate. However, when cells were exposed to LY294002 at the same time as the induction of differentiation (0 h), the myogenin expression was completely abrogated at both the mRNA and protein level after 24 h of incubation (Figure 2A). This result is consistent with previous reports on the activity of PI3-kinase during the induction of differentiation. In this study, we focused on the increased activity of PI3-kinase after the onset of differentiation (Figure 1B). At 40 h after inducing differentiation when myogenin expression had reached its peak, the cells were exposed to LY294002, and the expression of myogenin was examined at the indicated times. After reaching its peak at 40 h, the myogenin protein level slightly decreased during the differentiation process (Figure 2B). In contrast, the myogenin protein levels in the LY294002-treated cells were dramatically reduced, and the protein could barely be detected after 12 h of incubation. Of particular interest was the observation that the mRNA level was unaltered at any time-point, irrespective of whether the cells were incubated with LY294002. Both the protein and mRNA levels of GAPDH, a housekeeping gene, did not change during the differentiation process. These results suggest that the inhibition of myogenin protein accumulation by LY294002 in differentiating myoblasts is mediated by a post-transcriptional mechanism, rather than through a transcriptional mechanism.

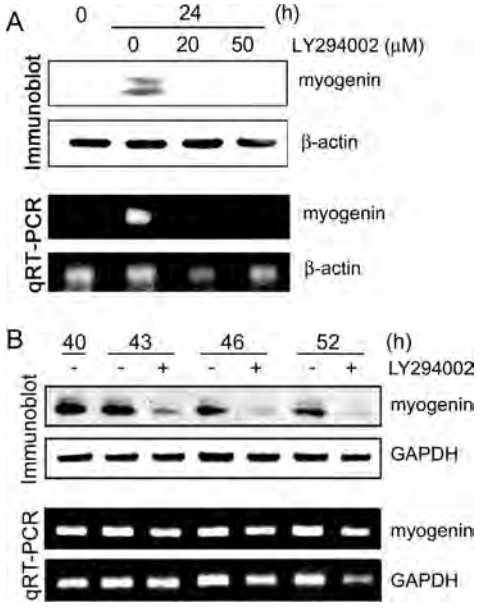


Figure 2. PI3-kinase regulates myogenin expression at both the post-transcriptional and transcriptional level. (A) At the time of inducing differentiation (0 h), the myoblasts were exposed to the indicated concentrations of LY294002 and cultured for 24 h. The cells were assayed for myogenin expression by Western blotting and qRT-PCR analysis. β-actin was used as a loading control for both experiments. (B) At 40 h after inducing differentiation, the cells were treated with (+) or without (−) 20 μM of LY294002 and cultured for the indicated times. The protein and mRNA levels of myogenin and GAPDH were determined using Western blotting and qRT-PCR analysis. GAPDH was used as a loading control. Results are representative of either three or four independent experiments.

### *LY294002 inhibits myogenin protein accumulation through a post-transcriptional mechanism rather than a transcriptional mechanism after differentiation has been induced*

The expression of protein can be regulated by changing the rate of protein synthesis and/or degradation. Abrogation of protein synthesis reduces the level of many proteins, especially those with short half-lives. We examined whether the level of myogenin protein was influenced by the rate of protein synthesis. Cells were exposed to cycloheximide at 40 h following the induction of differentiation, and the cells were further cultured for the indicated times. Cycloheximide inhibits the initiation, elongation and termination stages of protein synthesis by targeting the 60S ribosomal subunit (Edwards and Mahadevan 1992), so this agent has been routinely used to determine the half-lives of proteins (Song et al. 1998; McVean et al. 2000). Cycloheximide treatment dramatically reduced the amount of myogenin protein in a time-dependent manner in normal cultured cells (Figure 3A). Our results indicated that the half-life of myogenin protein was approximately 3 h. In contrast, GAPDH protein levels were not affected by cycloheximide at all tested time-points. As expected, the myogenin mRNA levels were not affected by cycloheximide. Next, we examined the half-life of myogenin mRNA by treating the cells with actinomycin D, which prevents transcription by inhibiting the function of RNA polymerase. We compared the half-life of myogenin mRNA to that of GAPDH, which is relatively stable. Cells were exposed to actinomycin D with or without LY294002 at 40 h after inducing differentiation, and the mRNA levels were measured using qRT-PCR. The myogenin mRNA levels gradually decreased when transcription was blocked with actinomycin D (Figure 3B). The half-life for myogenin mRNA was determined to be approximately 7 h. LY294002 did not affect the reduction in myogenin mRNA levels; therefore, the stability of myogenin mRNA was not affected by LY294002 after differentiation had been induced. These results clearly demonstrate that the myogenin protein was more unstable than its mRNA and suggest that the accumulation of myogenin is regulated by a post-transcriptional process.

Since protein synthesis can be regulated by the modulation of translation factors, we first examined whether LY294002 affected the activities of eEF2 or

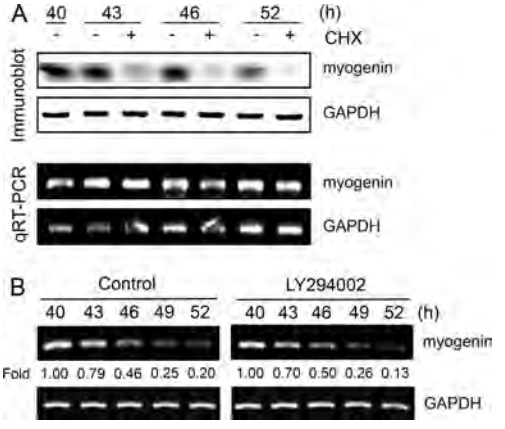


Figure 3. Measurement of myogenin protein and mRNA stability. (A) At 40 h after inducing differentiation, the cells were treated with (+) or without (−) 10 ng/ml of cycloheximide and cultured for the indicated times. The protein and mRNA levels of myogenin and GAPDH were determined by Western blotting and qRT-PCR analysis. GAPDH was used as a loading control. (B) To determine mRNA stability, the cells were incubated with 2 μg/ml of actinomycin D in the presence or absence of LY294002 (20 μM) at 40 h after inducing differentiation and cultured for the indicated times. The mRNA level of myogenin was determined by qRT-PCR analysis. GAPDH was used as a loading control. Results are representative of either three or four independent experiments.

eEF2K, which is the only kinase that phosphorylates eEF2. We have previously reported that PI3-kinase activity is closely related to the activity of eEF2, which is regulated by phosphorylation (Woo and Kim 2006). Whereas the translational activity of eEF2 is inhibited by phosphorylation (Ryazanov and Davydova 1989), eEF2K is activated by phosphorylation at Ser366. This facilitates the dephosphorylation of eEF2, which can then promote the activation of protein synthesis (Knebel et al. 2001; Wang et al. 2001). Phosphorylation of eEF2 (Ⓟ-eEF2) greatly increased with increasing doses of LY294002, whereas the eEF2 protein level was slightly reduced (Figure 4A). In contrast, the phosphorylation of eEF2K (Ⓟ-eEF2K) was substantially blocked by LY294002. Next, we examined whether LY294002 influenced the activity of other important translation factors, including $p70^{S6K}$, S6, 4E-BP1 as well as eEF2. The activities of these factors were examined by assaying their phosphorylation. The activities of all these factors, with the exception of eEF2, are stimulated by phosphorylation at the indicated sites (Ruvinsky and Meyuhas 2006). As shown in Figure 4B, phosphorylation of all the observed factors, except eEF2, was blocked by LY294002. Therefore, their activities were inhibited by LY294002, suggesting that inhibition of PI3-kinase activity results in abrogation of protein synthesis.

Next, we examined whether LY294002 influenced the rate of protein synthesis using metabolic labeling assays. Myoblasts cultured in differentiation medium with the indicated concentrations of LY294002 for 24 h were exposed to [$^{35}$S]Met for 1 h. Equal amounts of proteins were separated using SDS-PAGE and incorporation of [$^{35}$S]Met into the total protein population was determined by exposing the gel to X-ray film. LY294002 reduced [$^{35}$S]Met incorporation in a dose-dependent manner (Figure 4C). There was less than 50% incorporation in the cells treated with 50 μM of LY294002, as compared to untreated cells. The autoradiography data indicated that LY294002 inhibited [$^{35}$S]Met incorporation in the majority of proteins and not just a specific fraction (data not shown). These results clearly demonstrate that PI3-kinase is required for protein synthesis, because it regulates the activities of translation factors after differentiation has been induced.

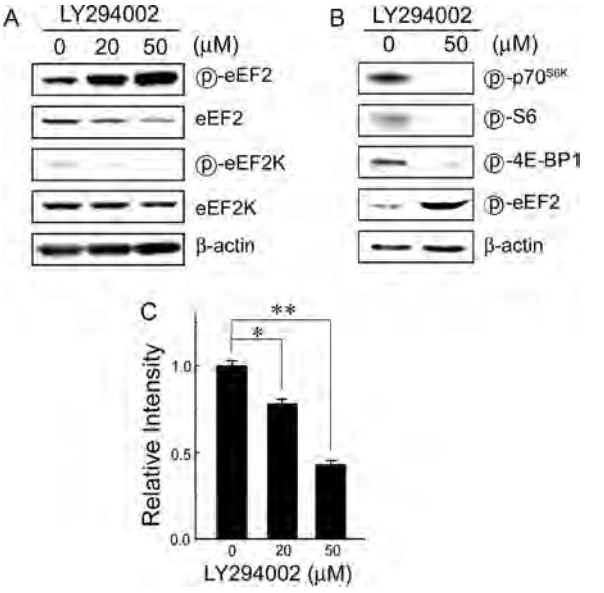


Figure 4. LY294002 blocks the activities of translational components in myoblasts after induction of differentiation. (A) At 40 h after inducing differentiation, the cells were exposed to the indicated doses of LY294002 and cultured for a further 6 h. The phosphorylation status of eEF2 and eEF2K was assayed using Western blotting with the corresponding antibodies. (B) The phosphorylation status of the indicated translation factors, including $p70^{S6K}$, S6, 4E-BP1 and eEF2, was assayed using Western blotting with the corresponding antibodies. β-actin was used as a loading control. Results are representative of three independent experiments. (C) Myoblasts were exposed to [$^{35}$S]Met for 1 h in Met-free DMEM and cultured for a further 24 h, as described in the 'Materials and methods'. Equal amounts of proteins were separated by SDS-PAGE. The intensities of [$^{35}$S]Met-labeled proteins were measured using the Scion Image program. Results are expressed as the ratio of the band intensity, as compared to the untreated control cells. Values represent the mean ± SEM of three independent experiments (*$P < 0.05$ and **$P < 0.01$).

## Discussion

Until recently, investigation of the function of PI3-kinase in the regulation of myogenesis has primarily focused on its regulation of myogenin transcription during the induction of differentiation. In this study, we demonstrate that PI3-kinase is also required for the sustained expression of myogenin throughout myogenesis. We determined that LY294002 blocked myoblast fusion even after cells had committed to differentiation, and PI3-kinase activity increased as myogenesis progressed (Figure 1). Based on these observations, we reasoned that PI3-kinase is required for the maintenance of myogenesis, in addition to its established role in the initial induction of differentiation. We focused our study on the regulation of myogenin expression by PI3-kinase after differentiation was induced. Our results indicate that PI3-kinase activity is related to the sustained expression of myogenin throughout myogenesis; however, the mechanism of this increased expression differs depending on the stage of differentiation. In the early stages, i.e., at the time of differentiation induction, PI3-kinase primarily regulates the transcription of myogenin, whereas it regulates the level

of myogenin through a post-transcriptional mechanism after differentiation has been induced.

The primary conclusion of this study is that PI3-kinase controls myogenin expression by predominantly regulating protein synthesis. Inhibition of PI3-kinase reduces protein synthesis and slows cell cycle progression (Alvarez et al. 2003). The protein synthesis is regulated at the initiation and/or elongation stages of translation (Proud 2004). PI3-kinase signaling is regulated by insulin or insulin-like growth factor (IGF)-1 (Shen et al. 2005), which both influence the activity and phosphorylation of $p70^{S6K}$, 4E-BPs and eEF2 via the induction of mammalian target of rapamycin (mTOR) (Wang et al. 2000). $p70^{S6K}$ phosphorylates S6, which plays a key role in initiation and/or elongation (Hay and Sonenberg 2004). Eukaryotic initiation factor 4E (eIF4E) recruits ribosomes to mRNAs, and its activity is inhibited by binding to hypo-phosphorylated 4E-BPs (Hay and Sonenberg 2004). Translation elongation requires the activity of eEF2, which catalyzes the translocation of the peptidyl-tRNA from the A to the P site of the ribosome (Browne and Proud 2002). In the previous report, we demonstrated that PI3-kinase is involved in withdrawal from the cell cycle during the early stages of differentiation, which is a prerequisite for the terminal differentiation of myoblasts. This process is regulated by the phosphorylation status of eEF2 (Woo and Kim 2006). In this study, we demonstrated that LY294002 inhibited all of these translation factors, including eEF2, by altering their phosphorylation status after differentiation was induced. This finding suggests that PI3-kinase-mediated signaling can regulate translation in both differentiating and proliferating cells.

In the LY294002-treated cells, the myogenin protein is rapidly depleted with no significant effect on the mRNA level (Figure 2B). Myogenin protein, similarly to other MRFs, has a relatively short half-life, which we determined to be less than 3 h (Figure 3A). In contrast, the half-life of the myogenin mRNA is longer, approximately 7 h (Figure 3B). These results are consistent with previous reports (Thayer et al. 1989; Edmondson et al. 1991; Figueroa et al. 2003; Viñals and Ventura 2004). Constant transcription and translation are required for the maintenance of a constant myogenin protein level during myogenesis. LY294002 impaired the activities of translation factors, so there was insufficient myogenin protein available during myogenesis due to the inhibition of protein synthesis, so the level of mRNA transcription was insignificant. The inhibition of the activities of translation components and the impaired rate of protein synthesis suggest that LY294002 blocks total protein synthesis rather than a subset of selected proteins. However, in contrast to myogenin, the protein level of GAPDH was not affected by exposure to LY294002. We suggest that this outcome is due to the difference in their half-lives; GAPDH has a much longer half-life than myogenin (Figure 3A). Because half-lives of both β-actin and GAPDH have been reported as longer than 30 h (Wilson et al. 1997; Franch et al. 2001; Herbert et al. 2001), they are widely used as controls. The mRNA and protein levels of these abundant proteins were not affected by LY294002 under our experimental conditions.

Myogenin can be degraded through the ubiquitin-proteasome pathway (Viñals and Ventura 2004). We examined whether LY294002 affected proteasome activity in myoblasts; however, there was no effect of LY294002 on proteasome activity under our experimental conditions (data not shown). To our knowledge, there have been no studies reporting that LY294002 directly regulates proteasome activity. Therefore, the LY294002-mediated reduction in myogenin protein levels is not due to increased degradation by the proteasome. It has been reported that myogenin degradation is promoted by the increased expression of Inhibitor of DNA binding/differentiation1 (Id1) (Viñals and Ventura 2004). Both the mRNA and protein levels of Id1 are reduced upon the induction of differentiation (Benezra et al. 1990). Since LY294002 was reported to inhibit the down-regulation of Id1 mRNA levels (Kaliman et al. 1996; Lopez-Carballo et al. 2002), we cannot exclude the possibility that the LY294002-mediated reduction in myogenin protein was due to a reduction in Id1 levels.

In this study, we used LY294002 as a PI3-kinase inhibitor. Although LY294002 has been widely used as a PI3-kinase-specific inhibitor in many researches, we could not fully exclude a potential problem of using the agent. Recently, several reports have shown that LY294002 might function through PI3-kinase-independent mechanism (Gharbi et al. 2007; Sun et al. 2008). In the previous report, we used wortmannin as well as LY294002 as PI3-kinase inhibitors (Woo and Kim 2006), but wortmannin was excluded in this study because it is known to inhibit other enzymes (Nakanishi et al. 1992; Yano et al. 1993) and it is very unstable in an aqueous solution (Vanhaesebroeck and Waterfield 1999; Franch et al. 2002). A more delicate system for compensating the possible problem(s) of using the chemical should be developed. In conclusion, we have shown that PI3-kinase functions as a key regulatory signaling molecule throughout the entire process of myogenesis, as it promotes the maintenance of myogenin expression. The mechanism of action for the effect of PI3-kinase on myogenin expression differs depending on the stage of differentiation. We demonstrate that PI3-kinase functions in both the initial increase in myogenin expression

through a transcriptional mechanism during the induction of differentiation and also contributes to the accumulation of myogenin protein after the induction of differentiation through post-transcriptional mechanisms. Collectively, these functions result in the maintenance of myogenin expression during myogenesis.

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