Glucocorticoids abate p70S6k and eIF4E function in L6 skeletal myoblasts

O. JAMEEL SHAH, SCOT R. KIMBALL, AND LEONARD S. JEFFERSON
Department of Cellular and Molecular Physiology, The Pennsylvania State University
College of Medicine, Hershey, Pennsylvania 17033

Received 14 October 1999; accepted in final form 27 January 2000

Shah, O. Jameel, Scot R. Kimball, and Leonard S. Jefferson. Glucocorticoids abate p70S6k and eIF4E function in L6 skeletal myoblasts. Am J Physiol Endocrinol Metab 279: E74–E82, 2000.—The catabolic properties of glucocorticoid hormones are largely attributable to dual regulation of protein degradation and synthesis. With regard to the latter, glucocorticoids modulate the translational machinery, namely that component functional in translation initiation. This investigation revealed that in L6 myoblasts, dexamethasone, a synthetic glucocorticoid, deactivated the ribosomal protein S6 kinase (p70S6k) within 4 h, as evidenced by diminished phosphorylation of its physiological substrate, the 40S ribosomal protein S6. This deactivation correlated with dephosphorylation of p70S6k at Thr389, whereas phosphorylation of Ser411 was unaffected. Furthermore, glucocorticoid administration induced dephosphorylation of the cap-dependent translational repressor, eukaryotic initiation factor 4E (eIF4E), thereby facilitating con-junction of the inhibitor and eIF4E. The mechanism of action is reminiscent of classical transcriptional regulation by steroid hormone receptors in that these effects were preceded by a temporal lag and were sensitive to inhibitors of glucocorticoid receptor function as well as transcriptional and translational inhibition. Okadaic acid and calyculin A corrected the dexamethasone-induced dephosphorylation of p70S6k and 4E-BP1, implicating a PP1- and/or PP2A-like protein phosphatase(s) in the observed phenomena. Hence, glucocorticoids attenuate distal constituents of the phosphatidylinositol-3 kinase signaling pathway and thereby encumber the protein synthetic apparatus.

eIF4E binding protein; eIF4G; dexamethasone; translation initiation

THE PATHOPHYSIOLOGICAL SEQUELAE that derive from hypercortisolism, as observed in Cushing’s syndrome or chronic glucocorticoid therapy, are often characterized by a marked loss of lean body mass caused by enhanced proteolysis (10, 27, 34) and/or diminished protein synthesis (29, 30). Moreover, glucocorticoids are inherently diabetogenic, in that hormonal excess renders peripheral tissues insulin resistant and thus refractory to the metabolic and anabolic processes governed by insulin despite associated hyperinsulinemia. At the cellular level, the mechanisms dictating the catabolic and antianabolic character of glucocorticoids are only now emerging.

Manifestation of the antianabolic effects of glucocorticoids is heralded by an initial series of biochemical events that predispose the cell to nitrogen loss. This acute phase occurs within 4 h and is distinguished in part by attenuation of the cell’s protein synthetic apparatus. In particular, the eIF4 class of eukaryotic initiation factors, which coordinately regulate expression of the overwhelming majority of cellular mRNAs, i.e., 7-methylguanosylated mRNAs, is potently affected. Administration of dexamethasone induces dephosphorylation of eIF4E binding protein 1 (4E-BP1), which strengthens its affinity for eIF4E, competitively inhibits eIF4G-eIF4E coupling, and ultimately prevents assembly of the functional eIF4F holocomplex (33). Not surprisingly, the 70 kDa ribosomal protein S6 kinase (p70S6k), which lies downstream of phosphatidylinositol-3 kinase (PI-3 kinase) and appears to be subject to direct or indirect regulation by the mammalian target of rapamycin (mTOR) is simultaneously dephosphorylated in skeletal muscle challenged with dexamethasone. This serine/threonine protein kinase has been implicated in cell cycle progression across the G1 checkpoint (4, 9, 21) and in the translational selection of transcripts exhibiting a unique 5′ terminal oligopyrimidine (TOP) signature (16).

Because glucocorticoids negatively modulate protein synthesis, in part via downregulation of key translation factors, an attempt was undertaken to characterize mechanistically those events that underlie and contribute to this process. Furthermore, the influence of acute glucocorticoid action on signal transduction pathways previously implicated in regulation of p70S6k and 4E-BP1 was assessed.

EXPERIMENTAL PROCEDURES

Materials. Enhanced chemiluminescence (ECL) detection kits and donkey anti-rabbit and sheep anti-mouse horseradish peroxidase-conjugated IgG were purchased from Amersham Life Sciences. Polyvinylidene difluoride (PVDF) membranes were purchased from Bio-Rad. [35S]Easytag express protein-labeling mix was acquired from NEN Research Prod-
GLUCOCORTICOIDs ABATE p70s6K AND eIF4E FUNCTION

RESULTS

The function of glucocorticoids in catabolic and anabolic capacities has long been appreciated. To begin to address the underlying molecular phenomena associated with glucocorticoid-regulated protein metabolism, events involved in the assembly of eIF4F, the cellular machinery that facilitates recruitment of mRNA to the ribosome, was assessed. This mRNA binding complex is composed of several polypeptides, including eIF4E, eIF4G, eIF4A, and eIF4B; eIF4G, the largest subunit of the complex, serves as a docking cap of eukaryotic mRNA, with eIF4G is required for loading ribosomes onto the message. Therefore, the formation of the active eIF4F complex represents an important control point for the regulation of translation initiation.

The classical mechanism of glucocorticoid action involves migration of the transformed glucocorticoid receptor into the nucleus, where it physically recognizes specific DNA response elements and thereby transactivates or transrepresses target genes. However, inference of various transcriptional pathways by the liganded glucocorticoid receptor can occur independently of the DNA binding properties of the receptor. Such genomic regulation cumulatively serves to tailor cellular phenotype to environmental demands and is
typically preceded by a temporal lag because of the time requirement of mRNA and protein synthesis and/or degradation. Indeed, 2 h of incubation with dexamethasone was required for significant redistribution of total 4E-BP1 into hypophosphorylated species; this occurred concomitantly with disappearance of the γ- or hyperphosphorylated form of the protein (Fig. 1A). Furthermore, because 4E-BP1-γ is the only form of the inhibitor incapable of sequestering eIF4E, the loss of the γ-form implies that a larger proportion of total eIF4E should be bound by 4E-BP1, thereby limiting its availability. Not surprisingly, co-immunoprecipitation of 4E-BP1 with eIF4E revealed that eIF4E was increasingly coupled to 4E-BP1 in direct relation to the period of incubation with dexamethasone (Fig. 1B).

The PI-3 kinase signal transduction module that regulates, through phosphorylation, the efficacy of 4E-BP1 mutually affects the phosphorylation and thus the activation state of p70S6k. Predictably, in cells administered dexamethasone, p70S6k was markedly dephosphorylated; however, the glucocorticoid required ≥2 h to manifest significant effect. In cells deprived of serum for 24 h, p70S6k resolves into multiple readily detectable electrophoretic forms after SDS-PAGE, wherein increasing phosphorylation retards protein mobility. Presumably, it is the most slowly migrating species that represents the heavily phosphorylated and thus highly active form of the kinase (36). Although four electrophoretic species are readily apparent in serum-deprived cells, the form of slowest mobility represents only a small fraction of the entire p70S6k pool. In dexamethasone-administered cells, total p70S6k redistributed into relatively dephosphorylated species, as evidenced by an accumulation of the bulk (85%) of the enzyme into the most rapidly migrating form, designated α, whereas immunoreactivity corresponding to the highly phosphorylated enzyme was lost (Fig. 1C). The close temporal correlation between dephosphorylation of 4E-BP1 and p70S6k underscores the nature of coordinate regulation of these factors.

Because the proportion of p70S6k residing in the uppermost band, i.e., the highly phosphorylated form, represented only a small fraction of the total kinase, the physiological significance of reduced basal activation by glucocorticoids seemed questionable. To address this issue, S6 phosphorylation was determined with the use of an anti-phospho-S6 antibody. Interestingly, the levels of phospho-S6 declined in parallel with the dephosphorylation of p70S6k observed after addition of glucocorticoids. Within 4 h of drug administration, phosphorylated S6 was reduced to ∼50% of control values (Fig. 1D). Thus basal p70S6k activity maintains S6 in a nominally phosphorylated state that is further diminished by glucocorticoid-induced dephosphorylation/inactivation of the kinase. Although this is a reasonable explanation, the possibility that the decrement in immunoreactive phospho-S6 is caused by another S6 kinase or S6 phosphatase cannot be excluded. Whereas in serum-deprived CTLL-20 cells, 3 h of exposure to dexamethasone reduces p70S6k activity by 50%, in quiescent NIH3T3 fibroblasts, the drug is completely ineffectual, emphasizing the cell selectivity of p70S6k regulation by glucocorticoids (22).

Because glucocorticoids are known to modulate the expression of particular genes, it seemed plausible that glucocorticoid-induced dephosphorylation of both p70S6k and 4E-BP1 could reflect reduced expression of candidate upstream regulators. However, neither the expression of mTOR nor that of PKB/Akt proteins was affected by dexamethasone administration (Fig. 2). Because in serum-deprived cells phosphorylation of PKB at Ser473, which is essential for PKB activation, was undetectable, a comparison of the activation state of PKB in control and dexamethasone-treated cells was

---

**Fig. 1.** Glucocorticoids downregulate the phosphorylation states of ribosomal protein S6 kinase (p70S6k) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1). L6 myoblasts were deprived of serum for 24 h and then administered dexamethasone (Dex, 1 μM) for the indicated times. Cells were harvested, lysed, and Western blotted with anti-4E-BP1 (A), anti-p70S6k (C), or anti-phospho-S6 antibodies (D). In the case of p70S6k, the electrophoretic forms were quantitated and the data expressed as %total p70S6k in the hyperphosphorylated (HP), i.e., non-α-, forms. Arrows indicate relative positions of α- and HP-forms. Similarly, multiple electrophoretic forms of 4E-BP1 were quantitated and the data expressed as %total 4E-BP1 in the most slowly migrating form, “γ”. B: Cell lysates were immunoprecipitated with anti-eIF4E antibody. The immunoprecipitates were then electrophoresed and immunoblotted with anti-eIF4E or anti-4E-BP1 antibodies. The Western blots were quantitated and the data expressed as the ratio of 4E-BP1 to eIF4E in arbitrary units. Data represent ≥3 independent experiments; values are means ± SE. *P < 0.05, †P < 0.01 vs. untreated cells by Student’s t-test comparison.
not possible. However, whereas glucocorticoids acutely attenuate insulin-like growth factor (IGF)-I-stimulated phosphorylation of p70S6k and 4E-BP1, IGF-I-stimulated phosphorylation of PKB at Ser473 is refractory to inhibition by glucocorticoids, suggesting that the influence of these hormones is exerted downstream of or parallel to PKB (unpublished results). Furthermore, phosphorylation of eIF4E by the MAP kinase-integrating kinases (Mnks) is believed to enhance the affinity of eIF4E for the 5’ cap of mRNA and thus to facilitate translation initiation. Neither the expression nor the activation of the Erks and the p38MAPks, which activate Mnks and thereby promote eIF4E phosphorylation, were significantly influenced by glucocorticoids (Fig. 2, B-D).

To determine whether downregulation of eIF4E and p70S6k was truly caused by specific effects mediated by the glucocorticoid receptor, cells were coadministered substances known to perturb glucocorticoid receptor function. Pretreatment of cells with the heat shock protein 90 (hsp90)-specific compound geldanamycin completely prevented the glucocorticoid-induced effects on 4E-BP1 (Fig. 3A) and corrected dephosphorylation of p70S6k (Fig. 3B). This benzoquinone ansamycin antibiotic, via interaction with the hsp90 component of glucocorticoid receptor heterocomplexes, hinders the steroid binding capacity of the receptor, as well as facilitating its degradation (32). Mifepristone...
(RU486), a glucocorticoid analog that binds the glucocorticoid receptor with higher affinity than dexamethasone but fails to efficiently transform the receptor, exhibited antigenic properties similar to geldanamycin: it entirely abrogated dephosphorylation of 4E-BP1 while returning the phosphorylation state of p70S6k to that observed in cells administered only mifepristone (Fig. 3, A and B). An effect of p70S6k dephosphorylation after exposure to both geldanamycin and mifepristone was noted.

Besides a prerequisite temporal lag, sensitivity to pharmacological translational and transcriptional inhibitors is a defining characteristic of gene induction by steroid hormones. Therefore, emetine, puromycin, and cycloheximide, each of which impairs the translational apparatus, were evaluated for their ability to abrogate dephosphorylation of 4E-BP1 and p70S6k in response to dexamethasone. Although the inhibitors prevented the glucocorticoid-induced dephosphorylation of 4E-BP1 and p70S6k, each compound promoted phosphorylation of these factors when administered in the absence of hormone (Fig. 4, A and B, cycloheximide not shown). Moreover, the same observation was made after treatment with the transcriptional inhibitor actinomycin D (Fig. 5, B-E and Fig. 6, A and B). To assess potential nontranslational influences of these inhibitors on p70S6k and 4E-BP1 phosphorylation, a temporal correlation, or lack thereof, between effector phosphorylation state and protein synthesis inhibition was sought. Indeed, inhibition of protein synthesis by cycloheximide occurred concomitantly with phosphorylation of p70S6k and 4E-BP1 (Fig. 5, A-E); even the more gradual decline in protein synthesis engendered by actinomycin D temporally paralleled the phosphorylation state of these effectors. Modulated phosphorylation of 4E-BP1 and p70S6k appears to be secondary to translational and not transcriptional inhibition per se, because actinomycin D, which presumably inhibits transcription as a prelude to translational inhibition, did not affect p70S6k and 4E-BP1 phosphorylation states before inhibition of protein synthesis.

It is noteworthy that dephosphorylation of p70S6k subsequent to treatment with glucocorticoids correlated with dephosphorylation of Thr389, thus supporting the premise that modification of this site accounts for the observed diminution in S6 phosphorylation (Figs. 6D and 1D). It is generally believed that phosphorylation of four key residues clustered in the carboxy-terminal autoinhibitory pseudosubstrate domain of p70S6k (Ser411, Ser418, Thr421, and Ser424), which relieves intrasteric occlusion of the kinase’s catalytic pocket, is induced after mitogen stimulation (12). These residues are flanked by a proline in the +1 position, rendering these sites, in vitro, sensitive to phosphorylation by enzymes of the MAP kinase superfamily, as well as other kinases exhibiting proline-directed substrate specificity. Curiously, phosphorylation of Ser411 was unchanged after exposure to glucocorticoids. Alternatively, actinomycin D (Fig. 6C) and okadaic acid (not shown), which promote phosphorylation of the kinase, induced a mild dephosphorylation of this site (Fig. 6C) concomitant with phosphorylation of Thr389 (Fig. 6D). Furthermore, phosphorylation of Ser411 was refractory to dephosphorylation by rapamycin (Fig. 6C), whereas neighboring residues Thr421 and Ser424 are reportedly much more sensitive to the macrolide (36). Importantly, dexamethasone does not reduce the phosphorylation status of Thr389 in cells treated with either actinomycin D (Fig. 6D) or okadaic acid (not shown) below that observed in cells administered inhibitor alone.

There is an increasing body of circumstantial evidence implicating a protein phosphatase in the mediation of signals propagated by mTOR [(15, 36) and reviewed in (5)]. Therefore, the protein phosphatase-1 and -2A (PP1 and PP2A) inhibitors, okadaic acid and calyculin A, were evaluated for their ability to correct the dephosphorylation of p70S6k and 4E-BP1 induced by dexamethasone. Indeed, both okadaic acid and calyculin A sufficiently reversed dephosphorylation of p70S6k (Fig. 7) and 4E-BP1 (not shown) in response to glucocorticoids. In fact, okadaic acid alone potentially

![Fig. 4. Protein synthesis inhibitors reverse glucocorticoid-induced dephosphorylation of 4E-BP1 and p70S6k. Serum-deprived L6 myoblasts were incubated in the presence of Dex (1 μM), emetine (1 μM), and/or puromycin (10 μM) for 4 h as indicated. A: cell lysates were separated by SDS-PAGE and Western blotted with an anti-4E-BP1 antibody. The electrophoretic forms were quantitated and expressed as %4E-BP1 of control. B: cell lysates were separated by SDS-PAGE and immunoblotted with anti-p70S6k antibody. Multiple electrophoretic forms were quantitated and expressed as %p70S6k in HP forms. Results are typical for ≥3 independent experiments.](http://ajpendo.physiology.org/doi/abs/10.1152/ajpendo.00451.2017)
induced phosphorylation of these effectors (not shown), implying that a PP1 and/or a PP2A-type phosphatase(s) either directly or indirectly regulates the phosphorylation states of p70S6k and 4E-BP1. Although both okadaic acid and calyculin A completely reversed the glucocorticoid-induced dephosphorylation of p70S6k and 4E-BP1, this was not due to inhibition of newly induced conventional phosphatases, because the relative expression levels of PP1 and PP2Ac, the catalytic subunit of PP2A, was not appreciably altered by glucocorticoids (not shown).

**DISCUSSION**

The translational effectors p70S6k and eIF4E comprise a translational unit distinguished by its sensitivity to both the fungal metabolite wortmannin and the immunosuppressive antibiotic rapamycin, which inhibit the phosphotransferase activities of PI-3 kinase and mTOR, respectively. The synthesis of D3-phosphorylated phosphoinositides at the plasma membrane by PI-3 kinase recruits pleckstrin homology domain-containing proteins such as the phosphoinositide-dependent kinase 1 (PDK1) and PKB. It is believed that this colocalization at the membrane promotes efficient phosphorylation of PKB by PDK1, whose activity appears to be constitutive. PDK1 also phosphorylates p70S6k at a site that is analogous to that phosphorylated on PKB (1, 26) and that is indispensable for S6 kinase activity (24). Although initial studies utilizing a membrane-localized form of PKB implied that this kinase was upstream of p70S6k, recent evidence suggests that such PKB-mediated activation of p70S6k is artifactual and in fact derives from constitutive membrane association rather than from bona fide substrate selectivity (8). The phosphorylation status of 4E-BP1, however, does appear to lie downstream of PKB, because a dominant-interfering PKB variant prevents the phosphorylation of 4E-BP1 but not p70S6k (8). Moreover, PKB phosphorylates mTOR on Ser2448, a site phosphorylated in response to insulin (23). Therefore, because both PKB and mTOR kinase activities are enhanced in response to insulin, and because mTOR-mediated 4E-BP1 phosphorylation is enhanced upon selective activation of a PKB/estrogen receptor chimera (31), a signal propagated via a PI-3 kinase/PKB/mTOR pathway is likely to regulate 4E-BP1 and thus eIF4E. However, it should be emphasized that accumulating evidence suggests that direct regulation of 4E-BP1 by mTOR is insufficient to explain 4E-BP1 phosphorylation observed in vivo (11, 13). At least in vitro, mTOR can phosphorylate p70S6k on both Thr389 and on the carboxy-terminal cluster of proline-directed
sites and does so cooperatively with PDK1 (14). Evidence has also emerged that mTOR may regulate PP2A and thereby exert an indirect effect on the phosphorylation status of p70S6k (25). Although TOR control of PP2A is better understood in yeast (15), evidence for a similar mechanism in mammalian cells remains largely circumstantial.

Despite the obvious complexity of regulation of p70\textsuperscript{S6k} and 4E-BP1, the data reported herein support reasonable conclusions regarding the modulation of this translational component by glucocorticoids. If glucocorticoids are transcriptionally affecting distal PI-3 kinase effectors, then the event(s) that determines their respective phosphorylation states is caused by induction or repression or both. At present, the nature of such transcriptional phenomena is uncertain. It is clear, however, that abrogation of the influences of glucocorticoids by actinomycin D, cycloheximide, emetine, and puromycin is related to translational inhibition. Although the transcriptional inhibitor actinomycin D augments both p70\textsuperscript{S6k} and 4E-BP1 phosphorylation, it does not do so until protein synthesis is attenuated (Fig. 5), suggesting that the resultant p70\textsuperscript{S6k} and 4E-BP1 phosphorylation states are not due to artifactual activation of upstream signal transduction pathways; rather, a signal emitted by the crippled translational machinery may negatively, but futilely, feed back to p70\textsuperscript{S6k} and 4E-BP1. Indeed, the notion of translational regulation by feedback mechanisms has been suggested. For instance, overexpression of eIF4E in a tetracycline-inducible background reduces p70\textsuperscript{S6k} and 4E-BP1 phosphorylation in proportion to the degree of eIF4E expression (17). Because the activation state of PKB, an upstream regulator of mTOR, was unaffected by eIF4E induction, it was proposed that a sensor for translational homeostasis lies downstream of PKB. Nevertheless, correction of glucocorticoid-induced dephosphorylation of p70\textsuperscript{S6k} and 4E-BP1 by transcriptional and translational inhibitors appears to be contingent on protein synthetic inhibition.

Because dexamethasone treatment results in accumulation of p70\textsuperscript{S6k}-\alpha, it is reasonable to speculate that this may be caused by inhibition of the initial series of phosphorylation events occurring within the carboxyl terminus of the kinase. All of these phosphorylation sites are situated in Ser/Thr, Pro motifs and, as such, are readily phosphorylated by MAP kinase and cyclin-dependent kinase family members in vitro. Whereas mTOR can also phosphorylate these residues in vitro (14), it is unlikely to be the physiologically relevant serum-activated kinase, because in an amino-terminal truncation background, phosphorylation of these clustered sites in response to serum is unabated by rapa-
mycin (7). Nevertheless, dephosphorylation of p70^{S6k} in response to dexamethasone is dissociable from dephosphorylation at Ser^{411} (Fig. 7C), because phosphorylation of this site is similar between control and dexamethasone-administered cells. Moreover, rapamycin does not influence the phosphorylation state of Ser^{411} in serum-deprived myoblasts. This finding is consistent with an earlier report demonstrating that in quiescent Swiss 3T3 cells, phosphorylation of this site is largely rapamycin insensitive (12), although serum-induced phosphorylation of Ser^{411} is abrogated by rapamycin pretreatment in the same cell system (24). Furthermore, although insulin enhances phosphorylation of Ser^{411}, this site remains substantially phosphorylated, even under conditions of serum starvation (36).

Interestingly, the same investigation revealed that cohort proline-directed sites, i.e., Thr^{421} and Ser^{424}, which lie within intimate proximity of Ser^{411}, are phosphorylated to a lesser extent in the basal condition and are more robustly phosphorylated after insulin stimulation. Thus, although putative proline-directed phosphorylation at Ser^{411} is unaffected by glucocorticoids, this does not preclude the possibility that glucocorticoids induce dephosphorylation of other neighboring residues in the enzyme’s carboxy-terminal tail.

It is also possible that glucocorticoids induce dephosphorylation of p70^{S6k} by hindering phosphorylation events that occur subsequent to those in the carboxy terminus. Of other sites associated with p70^{S6k} activation, i.e., Thr^{229}, Thr^{367}, Ser^{371}, Thr^{389}, and Ser^{404}, phosphorylation of Thr^{229}, which is situated in the catalytic activation loop, and of Thr^{389}, which lies within a hydrophobic segment interjacent to the catalytic loop and the carboxy terminus, cooperatively evokes full activation of the kinase (7, 36). Moreover, deletion of the amino and carboxy terminus, which renders the kinase resistant to inhibition by rapamycin, also reveals that phosphorylation of Thr^{229} persists in the basal state, whereas phosphorylation of Thr^{229} occurs only after mitogen stimulation (6). The Thr^{229} kinase has been identified as PDK1 (26), whose constitutive activation may explain perpetual phosphorylation of this site when the steric constraints of the amino and carboxy termini are alleviated by truncation. It is also noteworthy that phosphorylation of either Thr^{229} or Thr^{389} alone is associated with only 2–5% p70^{S6k} activity, whereas phosphorylation at both sites synergistically evokes complete activation. Under conditions of serum depletion, the hyperphosphorylated form of p70^{S6k} remains mildly discernible (Fig. 6B), as does basal phosphorylation of Thr^{389} (Fig. 6D); glucocorticoids render the corresponding signals undetectable. Furthermore, dexamethasone-induced dephosphorylation of Thr^{389} correlates with dephosphorylation of the endogenous p70^{S6k} substrate, ribosomal protein S6. Targeted deletion of p70^{S6k} in murine embryonic stem cells completely abolishes S6 phosphorylation, suggesting that p70^{S6k} is largely responsible for S6 phosphorylation in vivo (16). Therefore, dephosphorylation of S6 in response to glucocorticoids is likely to be mediated by dephosphorylation of p70^{S6k} at Thr^{389}.

The mechanism of dexamethasone-induced dephosphorylation of 4E-BP1 remains cryptic. It is clear, however, that the observed dephosphorylation is sufficient to promote sequestration of eIF4E by 4E-BP1 (Fig. 1B). The kinase activity associated with or intrinsic to mTOR phosphorylates 4E-BP1 in vitro and in transiently transfected HEK293 cells (2). Moreover, Thr^{37} and Thr^{46} are specifically targeted by mTOR, albeit the functional consequence of phosphorylation at these sites remains contentious (3, 11). Nonetheless, mTOR is a likely candidate for regulation of 4E-BP1 phosphorylation by glucocorticoids; if regulation of mTOR kinase activity does indeed occur, it is not via downregulation of the mTOR gene (Fig. 2A).

Glucocorticoids induce a clear redistribution of 4E-BP1 into α- and β-electrophoretic forms (Fig. 1A), both of which retain the capacity to associate with eIF4E (Fig. 1B). Because additional phosphorylation at other serum-responsive residues (e.g., Ser^{65} Ser^{70} Ser^{83} and Ser^{112}) requires previous phosphorylation of Thr^{37} and Thr^{46} the latter sites are proposed to prime 4E-BP1 for subsequent phosphorylation (11). It has been demonstrated that all three electrophoretic species of 4E-BP1 are detectable with the use of antiphosphopeptide antibodies specific for Thr^{37} and Thr^{46}, although the antibodies cannot discriminate between either site (11). Because 4E-BP1 exists predominantly in α- and β-forms after glucocorticoid administration, and because both α- and β-forms are phosphorylated at Thr^{37} and/or Thr^{46}, it seems plausible that glucocorticoids may attenuate the same serum-activated effectors that regulate the carboxy-terminal, serum-sensitive phosphorylation sites.

The distal element of the PI-3 kinase signal transduction cassette, comprised of mTOR, 4E-BP1, and p70^{S6k}, is recognized as a critical site of translational control in mammalian cells and, as such, has been the focus of widespread investigation. Herein, we report that glucocorticoids negatively regulate eIF4E and p70^{S6k} function in undifferentiated rat L6 skeletal myoblasts in a manner that appears characteristic of classical steroid hormone action in that the process requires several hours to emerge and is sensitive to transcriptional and translational inhibition. Furthermore, the close temporal correlation between modulation of 4E-BP1 and p70^{S6k} suggests that this mutual regulation may involve a common mechanism. Dephosphorylation of ribosomal protein S6 is secondary to dephosphorylation of p70^{S6k} at Thr^{389}, but not at Ser^{411}, suggesting that glucocorticoids deactivate p70^{S6k} through site-selective dephosphorylation.

This work was supported by National Institute of Health Grants DK-15658 (L. S. Jefferson) and T32-GM-08619 (O. J. Shah).

REFERENCES


