Ubiquitin (UbC) Expression in Muscle Cells Is Increased by Glucocorticoids through a Mechanism Involving Sp1 and MEK1*

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The muscle protein catabolism present in rats with insulin-dependent diabetes and other catabolic conditions is generally associated with increased glucocorticoid production and mRNAs encoding components of the ubiquitin-proteasome system. The mechanisms that increase ubiquitin (UbC) expression have not been identified. We studied the regulation of UbC expression in L6 muscle cells because dexamethasone stimulates the transcription of this gene and others encoding components of the ubiquitin-proteasome pathway. Results of in vivo genomic DNA footprinting experiments indicate that a protein(s) binds to Sp1 sites ~50 bp upstream from the UbC transcription start site; dexamethasone changes the methylation pattern at these sites. Sp1 binds to DNA probes corresponding to the rat or human UbC promoter, and treating cells with dexamethasone increases this binding. Deletion and mutation analyses of the rat and human UbC promoters are consistent with an important role of Sp1 in UbC induction by glucocorticoids. Dexamethasone-induced ubiquitin expression is blocked by mithramycin, an inhibitor of Sp1 binding. U0126, a pharmacologic inhibitor of MEK1, also blocks UbC transcriptional activation by dexamethasone; L6 cells transfected to express constitutively active MEK1 exhibit increased UbC promoter activity. Thus, glucocorticoids increase UbC expression in muscle cells by a novel transcriptional mechanism involving Sp1 and MEK1.

Skeletal muscle atrophy due to activation of the ubiquitin-proteasome proteolytic system is a common consequence of catabolic conditions (e.g. metabolic acidosis, chronic renal failure, cancer cachexia, sepsis, or acute diabetes) in experimental animals or patients (reviewed in Ref. 1). The program of responses to catabolic stimuli consistently includes increased levels of mRNAs encoding ubiquitin, ubiquitin-conjugating enzymes, and proteasome subunits. The increase in levels of mRNAs encoding ubiquitin and some of the proteasome subunits in atrophic muscle involves stimulation of transcription, at least in rats with chronic renal failure, acute diabetes, or sepsis (2–4), but there is little information about the mechanism(s) that enhance transcription of these genes. In rats with metabolic acidosis, acute diabetes, sepsis, or starvation, glucocorticoids are required for the rise in mRNAs encoding proteasome subunits and ubiquitin as well as the increase in protein degradation (3, 5–7). These earlier studies were carried out in adrenalectomized rats but the complexity of identifying signaling pathways in intact animals makes it difficult to ascertain how transcription of ubiquitin and other pathway genes is regulated by glucocorticoids or other signals.

In eukaryotes, a family of three genes (i.e. UbA, UbB, and UbC) encode protein products that are processed to yield free ubiquitin (8). The UbC mRNA is typically increased more than other ubiquitin mRNAs when there is evidence of muscle wasting in catabolic patients or when the ubiquitin-proteasome system is activated in muscle of catabolic rats (2, 9–14). The physiologic importance of the higher level of ubiquitin (UbC) mRNA is controversial because ubiquitin protein is present in the resting cell. However, in muscle of rats stimulated to degrade protein at an accelerated rate by starvation or denervation, Wing et al. found that the total content of ubiquitin (i.e. free ubiquitin and ubiquitin conjugated to proteins) was higher (15, 16). Since there was a concurrent increase in ubiquitin mRNA, it seems likely that enhanced UbC transcription is an integral aspect of the program of responses that results in muscle atrophy. Other evidence for increased ubiquitin utilization in muscles of rats with cancer, sepsis, or acute diabetes includes greater amounts of ubiquitin conjugated to muscle proteins compared with results in muscle of control rats (10, 17, 18).

Nenoi et al. (19) characterized the human UbC promoter region, and they identified potential binding sites for several transcription factors, including multiple Sp1 sites. We cloned the rat UbC promoter and noted conservation of many putative Sp1 binding sites between the rat and human promoters (20). Despite the stimulation of UbC transcription by glucocorticoids, neither the rat nor human UbC promoter sequence contains a consensus glucocorticoid-response element. To understand how glucocorticoids increase the expression of UbC in muscle, we explored potential signaling pathways that regulate UbC expression in L6 muscle cells and found evidence for involvement of Sp1 and the MEK1 pathway.

EXPERIMENTAL PROCEDURES

Expression and Reporter Plasmids—The human and rat UbC promoter sequences (GenBank™ accession numbers AF232305 and D63791, respectively) were amplified from genomic DNA (Roche Molecular Biochemicals) using PCR. Forward primers (Table I) and a human reverse primer 5'-CACGCTAGCAACTAGCTGTGCCACACCCG-3' or a rat reverse primer 5'-CAGCTAGCAACTAGCTGCGGGACGCGAG-3' were used. Both the forward and reverse primers contained a NheI restriction site at the 5'-end. A rat UbC DNA fragment (rUbCmут (~157) containing mutations in two Sp1 sites centered around ~50 bp was generated by PCR using a forward mutagenesis primer and its complementary reverse primer (Table I). All promoter DNA segments were subcloned into the pGL2-Basic firefly reporter plasmid (Promega, Madison, WI). A plasmid containing the thromboxane synthase minimal promoter (~90 to ~30) linked to the Renilla luciferase gene (pTS-
**Human and rat UbC promoter-firefly luciferase reporter constructs**

UbC DNA fragments were amplified by the polymerase chain reaction using the forward primer identified below and a species-appropriate reverse primer as described in under “Experimental Procedures.” Bases in rUbC (−137) that are different from the corresponding wild-type sequence are underlined.

| UbC DNA fragment     | Description   | Primer                                 |
|----------------------|---------------|----------------------------------------|
| hUbC (−655)          | Human; −655 to +3 | 5′-TACATTGCTCCTTTGTCGCCCGG-3′          |
| hUbC (−371)          | Human; −371 to +3 | 5′-TAAGGAAACCGGGCGGGCCAAA-3′          |
| hUbC (−279)          | Human; −279 to +3 | 5′-GCGTGCAGCTGACGAGA-3′               |
| hUbC (−243)          | Human; −243 to +3 | 5′-TGGATTCCTCGGCGGACG-3′              |
| rUbC (−340)          | Rat; −340 to +3   | 5′-GTATTAGCCTGCTTCATGGCA-3′           |
| rUbC (−137)          | Rat; −137 to +3   | 5′-GGCCGGAAATCCGGCCGTTGCGCCATGAC-3′   |
| rUbC mtUT (−137)     | Rat; −137 to +3, mutations in 2 overlapping Sp1 sites | 5′-GGCCGGAAATCCGGCCGTTGCGCCATGAC-3′   |

Note: The abbreviations used are: EGFP, enhanced green fluorescent protein; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; ERK, extracellular signal-regulated kinase.

RL served as a control for transfection efficiency (21). The SV40 promoter-firefly luciferase reporter plasmid, pGL2-Control, was purchased from Promega. Plasmids to overexpress Sp1 (pCGN-Sp1 (22)) and constitutively active MEK1 (pRaf-MEK1 (23)) were generously provided by Dr. T. Shenk (Princeton University) and Dr. N. Ahn (Howard Hughes Medical Institute, University of Colorado at Boulder), respectively. The expression vector pCMV-EGFP encoding the enhanced green fluorescent protein (EGFP) was purchased from CLONTECH (Palo Alto, CA).

**Cell Culture and Transient Transfections—**Rat L6 myocytes from the American Tissue Culture Collection (ATCC, Manassas, VA) were differentiated into myotubes by growing the cells to 70% confluence and then replacing the growth medium with Dulbecco’s modified Eagle’s medium supplemented with 2% horse serum (20). The medium was replenished every 2 days.

Transfection studies were performed with L6 myocytes and UbC promoter luciferase reporter plasmids as described (20). In all transfection experiments, the medium was replaced with Dulbecco’s modified Eagle’s medium supplemented with 2% horse serum after 24 h, and cells were maintained for 48 h before measuring luciferase activity. When the glucocorticoid responsiveness of the UbC promoter was tested, dexamethasone (100 nM) and/or dexamethasone (100 nM, 6 h) was added to the growth medium. DNase I digestion of the cytosolic extract was performed using 0.25 mM Tris-HCl (pH 6.8) and 2.7% SDS; the lysates were passed through an insulin syringe again. The expression vector pCMV-EGFP encoding the enhanced green fluorescent protein (EGFP) was purchased from CLONTECH (Palo Alto, CA).

**Nuclear Run-off Assays—**L6 myotubes were treated with or without dexamethasone (100 nM, 12 h) before total RNA was isolated using Tri-Reagent (Molecular Research Center, Cincinnati, OH). RNase protection assays were performed using total RNA (2 μg) and the rat UbC-specific and glyceraldehyde-3-phosphate dehydrogenase antisense riboprobes as described (26). Proteins (7 μg of nuclear extract or 20 μg of cytosolic extract) were separated by electrophoresis in a 7.5% polyacrylamide gel. Western blot analyses were performed with the anti-Sp1 antibody and the American Biosciences ECL detection system.

**In Vivo Genomic DNA Footprinting—**L6 myotubes were treated with or without dexamethasone (100 nM, 6 h). Nuclei from 1 × 10⁷ cells were isolated (27) and a run-off assay was performed as described (2).

**Western Blot Protein Analysis—**Western blot analyses for ubiquitin were performed after L6 myotubes were incubated with or without dexamethasone (1 μM). Some cells were treated with mithramycin (100 nM) and/or dexamethasone (1 μM) to study the effect of Sp1 in expression of ubiquitin protein. Cells were lysed in a buffer consisting of 83 mM Tris-HCl (pH 6.8) and 2.7% SDS; the lysates were passed through an insulin syringe to shear DNA. Glycerol and β-mercaptoethanol were added (final concentrations of 10 and 5%, respectively), and lysates were boiled for 5 min and passed through an insulin syringe again. Proteins (50 μg/lane) were separated by SDS-polyacrylamide gel electrophoresis in a 15% acrylamide gel and transferred to a nitrocellulose membrane that was then baked at 75 °C for 30 min. A rabbit polyclonal antibody (Sigma or Calbiochem) was used to detect free and protein-conjugated ubiquitin. Western blots were developed using ECL Plus (Amersham Biosciences), and luminescence was measured using a Storm PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA). At other times, autoradiography was performed.

**Glucocorticoids and Ubiquitin (UbC) Transcription**

**TABLE I**

| DNA fragment Description | Primer |
|--------------------------|--------|
| 5′-TACATTGCTCCTTTGTCGCCCGG-3′ | 5′-TAAGGAAACCGGGCGGGCCAAA-3′ |
| 5′-GCGTGCAGCTGACGAGA-3′ | 5′-TGGATTCCTCGGCGGACG-3′ |
| 5′-GTATTAGCCTGCTTCATGGCA-3′ | 5′-GGCCGGAAATCCGGCCGTTGCGCCATGAC-3′ |

1 The abbreviations used are: EGFP, enhanced green fluorescent protein; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; ERK, extracellular signal-regulated kinase.
Footprinting analysis of the antisense strand was less informative because there are only two guanine residues in this region. Both guanines were hypomethylated, relative to the in vitro methylated DNA (data not shown), but dexamethasone treatment did not change the extent of their methylation. These results (summarized in Fig. 2C) suggest that the Sp1 site(s) located ~50 bp upstream of the transcription start site of the rat UbC promoter is occupied in vivo and that its occupancy changes in response to dexamethasone treatment. Notably, there are multiple putative Sp1 binding sites in the first ~340 bp upstream of the transcription start sites of the rat and human UbC promoters, respectively (Fig. 2C).

Based on the results of the in vivo footprinting, we studied the transcription factor that binds to the putative Sp1 sites located ~50 bp upstream of the transcription start site in the rat UbC promoter using electrophoretic mobility shift assays. Treating L6 myotubes with dexamethasone increased the binding of a nuclear protein to a DNA probe that spans the Sp1 binding sites in the rat UbC promoter (i.e. corresponding to the positions where dexamethasone changed the in vivo methylation pattern; Fig. 3, lane 1 versus lane 2). Specificity of DNA-protein binding was demonstrated because the addition of a 100-fold excess of unlabeled rat UbC probe or consensus Sp1 competitor probe (containing three Sp1 sites) to the binding reaction prevented complex formation (Fig. 3, lanes 3 and 5). When the Sp1 sites in the rat UbC probe or the consensus Sp1 competitor probes were altered (i.e. rUbC mut2x), the competitor probes did not block formation of the protein-DNA complex (Fig. 3, lanes 4 and 6). Evidence that the nuclear protein that binds to the UbC probe is Sp1 included the following. 1) The complex was supershifted by an anti-Sp1 antibody (Fig. 3, lane 7); antibodies to AP-1 or the p50/p65 subunits of NF-kB did not react with the protein-DNA complex (data not shown). 2) Reconstituent Sp1 protein formed a complex with the UbC probe, and that complex migrated with a mobility indistinguishable from the endogenous L6 nuclear protein-DNA complex (Fig. 3, lane 9). 3) Mutating the two Sp1 sites in the rat UbC probe (~67 to +28) abolished its ability to form a DNA-protein complex with nuclear proteins isolated from dexamethasone-treated or control cells (Fig. 3, lanes 10 and 11).

To demonstrate the importance of Sp1 sites located in the rat UbC transcription start site to the dexamethasone response, we performed transfection studies with rat UbC promoter-firefly luciferase reporter plasmids. Previously, we demonstrated that dexamethasone stimulated luciferase activity by 250% in L6 cells transiently transfected with a rat UbC promoter-firefly luciferase reporter plasmid (rUbC (~340)) containing a segment of the rat UbC promoter from ~340 to +3 (+1 denotes the start transcription site); the steroid receptor antagonist, RU486, completely blocked this induction (20). A construct containing the ~137 to +8 segment of the rat UbC promoter (rUbC (~137)) showed the same level of induction of luciferase activity as rUbC (~340) (Fig. 4). When cells were transfected with rUbCmut (~137) containing changes in the two overlapping Sp1 sites located ~50 bp upstream of the start transcription site, there was significantly less induction of luciferase activity by dexamethasone as compared with rUbC (~137) (Fig. 4). Basal luciferase activity of rUbCmut (~137) (normalized for transfection efficiency) was also significantly decreased compared with the activity measured from cells transfected with rUbC (~137) (Fig. 4). These findings are consistent with the conclusion that Sp1 sites are important for both the basal expression of the UbC gene and its responsiveness to dexamethasone.

The human UbC promoter, like the corresponding rat promoter, contains several putative Sp1 binding sites, so we tested
whether glucocorticoids also stimulate its transcriptional activity in L6 cells. Dexamethasone significantly increased luciferase activity in cells transfected with constructs containing segments of the human UbC promoter extending upstream of \( 371 \), but this stimulation was diminished in cells transfected with plasmids containing a promoter segment between \( 279 \) to \( 4 \) or shorter (Fig. 5A). Tandem binding sites for Sp1 are located between \( 371 \) and \( 279 \) in the human UbC promoter, consistent with the conclusion that Sp1 is important for the induction of the human UbC promoter by dexamethasone.

Therefore, we performed electrophoretic mobility shift assays with nuclear extracts from cells treated with or without dexamethasone and a probe to the \( 319 \) to \( 280 \) region of the human UbC promoter. The results of these binding assays were identical to those we obtained with the rat UbC probe. (Fig. 5B). Thus, our findings using the rat UbC promoter apply to the human UbC gene, because dexamethasone increased Sp1 binding to this promoter.

To determine whether Sp1 regulates the activity of the UbC promoter, we transiently co-transfected L6 cells with either a rat (rUbC \((-340)\)) or human (hUbC \((-655)\)) UbC promoter-firefly luciferase reporter plasmid plus plasmids for expressing either enhanced green fluorescent protein (pCMV-EGFP as a control) or Sp1 (pCGN-Sp1). Overexpressing Sp1 increased basal firefly luciferase activity \( \sim 2 \)-fold above the level in control cells transfected with pCMV-EGFP (p < 0.05 versus control for each plasmid; Fig. 6A). We also tested whether mithramycin, an inhibitor of Sp1-DNA binding (24, 25, 31), could block the increase in luciferase reporter activity induced by glucocorticoids. When transfected cells were pretreated with mithramycin, the increase in luciferase activity was significantly decreased (Fig. 6B). Therefore, these findings indicate that Sp1 is an important transcriptional regulator of the UbC promoter in L6 cells.
cin, dexamethasone no longer induced the rat or human UbC promoters linked to the luciferase reporter gene (Fig. 6B).

Next we tested whether mithramycin would block the increased expression of ubiquitin protein (i.e. the levels of free ubiquitin and/or ubiquitin-protein conjugates) in response to dexamethasone. Treating control cells with mithramycin decreased the amount of free ubiquitin and ubiquitin-protein conjugates (Fig. 7). In cells treated with dexamethasone, mithramycin eliminated the increase in both forms of ubiquitin protein (Fig. 7). Some bands on the autoradiogram did not change with mithramycin treatment, whereas others, notably the high molecular weight ubiquitin-protein conjugates, were significantly decreased by mithramycin. This result suggests that the decrease in ubiquitin was not simply a toxic reaction because ubiquitin conjugation to specific proteins continued to occur. Furthermore, differences in the amounts of ubiquitin and ubiquitin-protein conjugates in cells treated with or without mithramycin were not due to cell death since treated cells excluded trypan blue to the same extent as control cells (less than 1% of cells in either group were stained by trypan blue). Thus, treatments designed to alter Sp1 function in L6 muscle cells produced predictable changes in the expression of ubiquitin protein and in transcription assays using UbC promoter-linked reporter plasmids.

Because Sp1 is generally considered to be a transcription factor that regulates many genes, an important question is whether dexamethasone increases Sp1 transactivation of other genes in L6 muscle cells. To test this possibility, we transfected L6 cells with the SV40 early promoter-firefly luciferase reporter plasmid, pGL2-Control because the SV40 promoter contains several binding sites for Sp1 but no glucocorticoid response element, and it has been used to measure Sp1-mediated transcriptional responses (32, 33). Dexamethasone stimulated luciferase activity 219 ± 5% in cells transfected with pGL2-Control; mithramycin blocked the response. We also tested...
whether dexamethasone changes the expression of Glut-1 glucose transporter protein, because this gene is regulated by Sp1 (34). In contrast to ubiquitin, we found no difference in the amount of Glut-1 protein between control and dexamethasone-treated cells (data not shown). These results suggest that in muscle cells, glucocorticoids selectively alter the expression of genes whose transcription involves Sp1.

Do glucocorticoids increase Sp1 expression in L6 muscle cells? We measured the levels of Sp1 in untreated and dexamethasone-treated cells by Western blot analysis and found no difference in the cellular levels of Sp1 (data not shown). We also investigated whether dexamethasone induced a translocation of Sp1 from the cytosol to the nucleus. Again, there was no difference in the amounts of Sp1 in either the cytosolic or nuclear fractions of control and dexamethasone-induced cells (Fig. 8). These findings suggest that dexamethasone treatment increases the ability of Sp1 to bind to the UbC promoter rather than simply increasing the amount of Sp1 protein in the nucleus.

The MAPK signaling pathway has been reported to stimulate Sp1-mediated transcription (35, 36), and some reports
have suggested that certain glucocorticoid-mediated responses are linked to the MAPK pathway (37, 38). Therefore, we tested whether U0126, a pharmacologic inhibitor of MEK1/2, would inhibit the transcriptional activation of the \( \text{UbC} \) promoter in L6 cells transfected with \( \text{rUbC} \) by dexamethasone. Basal luciferase activity in transfected cells pretreated with U0126 (100 nM) before adding dexamethasone (100 nM) was only 15% of the activity measured in control cells pretreated with U0124, an inactive analog of U0126 (Fig. 9A). Dexamethasone increased \( \text{UbC} \) promoter activity 3-fold in cells pretreated with U0124 but did not stimulate the promoter in cells treated with U0126. We confirmed that MEK1 can stimulate \( \text{UbC} \) transcriptional activity by co-transfecting cells with \( \text{rUbC} \) and a plasmid to express constitutively active MEK1 (R4F-MEK1). Basal luciferase activity was 3-fold higher in cells transfected with R4F-MEK1 compared with control cells transfected with a green fluorescent protein expression plasmid (Fig. 9B). Dexamethasone did not stimulate transcriptional activity further.

**DISCUSSION**

In rats with catabolic conditions causing muscle atrophy, glucocorticoids are required for the increase in both protein degradation and levels of mRNAs encoding components of the ubiquitin-proteasome proteolytic system, including \( \text{UbC} \) (5, 9, 39). Although neither the rat nor the human \( \text{UbC} \) promoter sequence contains a classical glucocorticoid response element, the dexamethasone-induced increase in \( \text{UbC} \) expression is mediated by the glucocorticoid receptor because the receptor antagonist, RU486, blocked the transcriptional response (20). We now provide evidence that Sp1 and MEK1 are important components of the glucocorticoid-induced mechanism that stimulates \( \text{UbC} \) expression in L6 muscle cells.

A combination of *in vivo* and *in vitro* techniques was used to demonstrate the importance of Sp1 in the regulation of \( \text{UbC} \) expression as follows. 1) *In vivo* genomic footprinting analysis of the rat \( \text{UbC} \) promoter in L6 cells suggested that dexamethasm...
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some increases the occupancy of Sp1 sites located upstream of the transcription start site. 2) In vitro DNA binding experiments confirmed that Sp1 binds to cognate sites in the rat and human UbC promoters and that dexamethasone increases this binding. 3) Mutating the Sp1 sites in a rat UbC promoter-luciferase minigene (rUbC (−137)) decreased its basal level of activity and prevented its induction by glucocorticoids. 4) Mithramycin prevented the dexamethasone-induced increase in UbC promoter activity as evaluated with an artificial reporter gene; mithramycin also decreased the endogenous levels of free ubiquitin and ubiquitin-protein conjugates in control cells and blocked the increase in ubiquitin content in L6 cells treated with dexamethasone. These findings indicate that Sp1 is important for both basal UbC expression and for induction of this gene by dexamethasone.

How do glucocorticoids increase Sp1-dependent transactivation of the UbC promoter? Sp1-dependent transcription can be regulated by increasing Sp1 protein (40); however, dexamethasone did not change either the cell content of Sp1 protein or the amount of Sp1 in the nucleus of L6 muscle cells. Instead, we found that the transcriptional response to dexamethasone involves the MEK kinase in the extracellular signal-regulated kinase (ERK) MAPK pathway, suggesting that Sp1 is a downstream target of ERK. Consistent with this conclusion, several reports indicate that Sp1 activity can be regulated positively or negatively by phosphorylation (36, 40–45), and in Sp1, there are numerous potential phosphorylation sites that are conserved across species. For example, nerve growth factor activates ERK2, which phosphorylates Sp1 in PC12 cells; this response inhibits Sp1 binding to the N-methyl-d-aspartate receptor 1 promoter (45). In human gastric carcinoma (ACS) cells, epidermal growth factor induces an ERK2-mediated phosphorylation of Sp1, but in this case, Sp1 binding to the gastrin promoter is increased (36). Thus, ERK and perhaps other kinases regulate Sp1 activity in a tissue-specific manner. In this regard, we have found that glucocorticoids stimulate UbC gene expression only in skeletal muscle cells. 2

If glucocorticoids increase UbC expression by stimulating Sp1 activity in muscle cells, does this mean that all Sp1-responsive genes are transactivated? The answer is no, because dexamethasone did not change the amount of Glut-1 glucose transporter in L6 cells although this gene is reported to be regulated by Sp1 (34). Others report that cyclin A-dependent kinase phosphorylates Sp1 in its N-terminal region in 3T3 cells and that two Sp1-regulated genes, dihydrofolate reductase and thymidylate synthetase, are concurrently transactivated by cyclin A-dependent kinase (41). In these same cells, the Sp1-responsive collagen type VII promoter is not stimulated by the cyclin-dependent kinase. Thus, Sp1 can be induced to regulate the transcription of genes selectively. Selectivity could be accomplished by inducing interactions between Sp1 and other transcription factors, and there is precedence for such interactions (28, 46–48). However, we did not find evidence for such interactions in L6 cells treated with dexamethasone because the major DNA-protein complex formed in mobility shift binding assays using the UbC probe had a mobility indistinguishable from the recombinant Sp1 protein-DNA complex. Still, we cannot exclude the possibility that Sp1 associates with another transcription factor that binds to a location in the UbC promoter that is not contiguous with Sp1 binding sites. An alternative mechanism is that phosphorylation of Sp1 increases its affinity for specific binding sites.

The relevance of the present findings is that glucocorticoids are required for the increase in mRNAs that encode many components of the ubiquitin-proteasome system in atrophic muscle. Our studies only address how glucocorticoids regulate the transcription of one system component, ubiquitin. In an earlier study, we found that glucocorticoids act by an entirely independent mechanism; glucocorticoids were found to antagonize a suppressive effect of NF-κB on transcription of the proteasome C3 subunit gene, leading to increased expression of this subunit (26). Clearly, the transcriptional response of the UbC gene involves a different mechanism. Our findings provide the first evidence that increased UbC transcription leads to higher levels of ubiquitin mRNA and protein in either a cell line or animal organ. The mechanism of UbC gene induction by glucocorticoids is novel because it involves Sp1 and the MEK1 MAPK pathway.

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