Glucocorticoids Induce Proteasome C3 Subunit Expression in L6 Muscle Cells by Opposing the Suppression of Its Transcription by NF-κB*

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Muscle wasting in catabolic conditions results from activation of the ubiquitin-proteasome proteolytic pathway by a process that requires glucocorticoids and is generally associated with increased levels of mRNAs encoding components of this proteolytic system. In L6 muscle cells, dexamethasone stimulates proteolysis and increases the amount of the proteasome C3 subunit protein by augmenting its transcription. Transfection studies with human C3 promoter-luciferase reporter genes and electrophoretic mobility shift assays revealed that a NF-κB protein complex containing Rel A is abundant in L6 muscle cell nuclei. Glucocorticoids stimulate C3 subunit expression by antagonizing the interaction of this NF-κB protein with an NF-κB response element in the C3 subunit promoter region. Dexamethasone also increased the cytosolic amounts of the NF-κB p65 subunit and the IκBα inhibitor proteins in L6 cells. Incubation of L6 cells with a cytokine mixture not only increased the amount of activated NF-κB but also decreased C3 promoter activity and lowered endogenous C3 subunit mRNA. Thus, NF-κB is a repressor of C3 proteasome subunit transcription in muscle cells, and glucocorticoids stimulate C3 subunit expression by opposing this suppressor action.

Protein degradation is a highly regulated cellular process that removes mutant, damaged, or misfolded proteins and transient signaling proteins, processes antigenic proteins, and supplies amino acids for protein synthesis and energy. There has been an explosion of interest in the ATP-dependent, ubiquitin-proteasome pathway because it degrades a variety of proteins in many cell types as well as the bulk of myofibrillar protein in muscle (reviewed in Ref. 1). In muscle, this pathway is stimulated in pathologic conditions associated with muscle atrophy (e.g. acidosis, uremia, diabetes, and cancer (1–6)). Inhibitors of lysosomal and calcium-dependent proteases do not block this accelerated muscle proteolysis, but inhibitors of the proteasome or ATP synthesis do, indicating that the ubiquitin-proteasome pathway is activated in catabolic states (3–9).

What signals activate the ubiquitin-proteasome pathway in muscle? Studies of rats with cancer or sepsis or normal rats treated with cytokines (e.g. tumor necrosis factor-α and interleukin-6) raise the possibility that inflammatory cytokines could be involved in initiating muscle catabolism (reviewed in Ref. 10). Metabolic acidosis is another condition that activates the ubiquitin-proteasome pathway in muscle, but the intracellular pH in the muscle of acidotic rats is only minimally lowered (3, 11), suggesting that acidosis stimulates protein degradation indirectly, possibly through the release of cytokines from macrophages (12). Insulin can also regulate protein degradation in muscle because conditions associated with a low insulin level (e.g. acute diabetes and fasting) stimulate protein degradation via the ubiquitin-proteasome pathway (5, 6, 13). Notably, we and others find that glucocorticoids are necessary for the catabolic response to other stimuli (e.g. acidosis, insulin deficiency, and sepsis) in animals and muscle cells, but their role is permissive unless pharmacological doses of glucocorticoids are given (1, 13–16).

When ubiquitin-proteasome proteolysis is accelerated in muscle, generally there are concurrent increases in the levels of mRNAs encoding pathway components (1). Using nuclear run-off assays, we showed there is increased transcription of ubiquitin and proteasome C3 subunit mRNAs in the muscles of rats with uremia or diabetes (4, 5). There may be a link between the levels of ubiquitin-proteasome pathway mRNAs and protein degradation because Davies and colleagues (17–19) reported that preventing transcription of the proteasome C2 subunit using antisense oligonucleotides reduces proteasome quantity, overall proteolytic activity of the proteasome, and protein degradation in liver and hematopoetic cells.

Here, we report studies of L6 cells derived from rat muscle as we found that dexamethasone or acidification stimulates protein degradation and transcription of the C3 subunit as in intact rats. Hence, we investigated the mechanism by which glucocorticoids influence expression the human proteasome C3 subunit promoter.

MATERIALS AND METHODS

Cell Culture and Intracellular Protein Degradation Measurements—Rat L6 muscle cells (American Type Culture Collection, Manassas, VA) were grown in Dulbecco’s modified Eagle medium containing N-[2-hydroxyethyl]-piperazine-N’-[2-ethanesulfonic acid] (Life Technologies, Inc.) and supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin in a humidified atmosphere consisting of 5% CO2/95% oxygen. Protein degradation in confluent monolayers of L6 cells was measured by the release of free [14C]phenylalanine from cell proteins prelabeled with 1-[14C]phenylalanine (15, 20, 21).

Nuclear Run-off Transcription Assays—L6 muscle cells were incubated in 2% horse serum with or without 50 nM dexamethasone for 6 h before isolating cell nuclei according to Groudine et al. (22). Run-off assays were performed as described (4–6).

Northern Blot Hybridizations—L6 cells were grown to confluence, and the medium was replaced with Dulbecco’s modified Eagle’s medium supplemented with 2% horse serum. Dexamethasone (50 nM) was added to some wells, whereas other cells were incubated at pH 7.1 for 12 h.
before purifying total RNA using TRIReagent (Molecular Research Center, Cincinnati, OH). Northern blot hybridizations were performed with a rat proteasome C3 subunit cDNA followed by a rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (15).

Expression and Reporter Plasmids—A human proteasome C3 subunit reporter fragment from −460 to +1 was amplified from a C3 genomic DNA clone fragment (23) using the polymerase chain reaction and cloned into the pGL2 Basic firefly luciferase reporter plasmid (Promega Corp, Madison, WI). Deletion reporter plasmids were prepared by amplifying shorter fragments of the C3 promoter by polymerase chain reaction and ligating them into the pGL2 Basic plasmid. A C3 promoter fragment containing mutations in the NF-κB-like motif at −322 to −313 (NF-κB−) was generated by polymerase chain reaction using a forward mutagenesis primer 5′-GACTAAGAATTTCAAGCGG- GCTCCTGAAGCCTCCTC-3′ (mutated bases are indicated in bold and underlined) and a reverse mutagenesis primer 5′-GGCTTGAATTTCTTGACTTCTGGTGTTGAAA-3′; the resulting DNA was ligated into the pGL2 Basic plasmid.

pCMV-p65, an expression plasmid containing the NF-κB p65 protein-coding sequence under the transcriptional control of the cytomegalovirus (CMV) promoter and pCMV-p65/50, an expression vector encoding a p65/p50 chimeric protein composed of the DNA binding domain (amino acids 1–370) of p50 and the transcriptional activation domain (amino acids 309–550) of p65, were generously provided by Dr. C. Rosen (Roche Instutitionen, NJ) (24). Expression plasmids encoding mutant forms of IκB (pCMV IκB K21/22R and pCMV IκB ΔN14) were generously provided by D. W. Ballard (Vanderbilt University, Nashville, TN (25)).

Cell Transfections—L6 monolayers (40% confluence) were transfected with proteasome C3 subunit promoter-luciferase reporter gene plasmids using the Fugene-6 transfection reagent (Roche Molecular Biochemicals). Cells were cotransfected with pRL-TK, a plasmid containing the Renilla luciferase gene under the transcriptional control of the cytomegalovirus (CMV) promoter and pCMV-p65/50, an expression vector encoding a p65/p50 chimeric protein composed of the DNA binding domain (amino acids 1–370) of p50 and the transcriptional activation domain (amino acids 309–550) of p65, were generously provided by Dr. C. Rosen (Roche Instutitionen, NJ) (24). Expression plasmids encoding mutant forms of IκB (pCMV IκB K21/22R and pCMV IκB ΔN32/36) were generously provided by D. W. Ballard (Vanderbilt University, Nashville, TN (25)).

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear were harvested from confluent L6 cell monolayers after incubation with 50 nM dexamethasone for periods of 20 min to 24 h; control cells were treated with vehicle only. Nuclear protein extracts were prepared according to Dignam et al. (26). The sequence of the “sense” strand of each double-stranded oligonucleotide probe was: NF-κB−, 5′-GAGGACTGGGAAA-TAGAATTCTGGCCTCGGAGACCTCCTC-3′; NF-κB+, 5′-AGTTGGAGGGGACTTTCCCAGGC-3′. Binding experiments contained 0.5–0.9 ng of 32P-labeled annealed oligonucleotide (105 dpm), 5 μg of nuclear proteins, 10 mM Tris-Cl (pH 7.5), 100 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 5 mM MgCl2, 5% (v/v) glycerol, and 5 μg of poly(dI-dC) and were incubated at 20 °C for 20 min. When competitor DNA or antibodies (e.g. polyclonal antibodies that recognize NF-κB p65, Santa Cruz Biotechnology, Santa Cruz, CA) were included in the binding reaction, the reactions without labeled DNA were incubated at 20 °C for 20 min; after the 32P-labeled DNA probe was added, the reactions were incubated for an additional 20 min at 20 °C. Reaction products were separated in 2.5% glycerol, 4% polyacrylamide gels with a buffer containing 44.5 mM Tris, 44.5 mM boric acid, and 1 mM EDTA.

Western Blot Protein Analysis—L6 cells were grown to confluence, and the medium was replaced with Dulbecco’s modified Eagle’s medium supplemented with 2% horse serum; when glucocorticoids were studied, cells were incubated with dexamethasone (50 nM). Control and treated cells were lysed, and nuclear protein extracts were prepared according to Dignam et al. (26); the supernatant obtained after sedimenting the intact nuclei was used as the source of cytosolic proteins. Nuclear and/or cytosolic proteins (20 μg of protein/lane) were separated by SDS-polyacrylamide gel electrophoresis in a 10% acrylamide gel and transferred to a polyvinylidene difluoride membrane. The polyclonal antibodies used to detect IκBα or NF-κB p65 subunit proteins were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Western blots were reacted with 1:5,000 dilution of primary antibodies and a 1:10,000 dilution of horseradish peroxidase-conjugated secondary antibody; blots were developed using enhanced chemiluminescence technology.

To compare the levels of proteasome C3 subunit protein in control and dexamethasone-treated L6 muscle cells (50 nM, 24 h), cells were lysed in Laemmli sample buffer, and total cell protein (20 μg of protein/lane) was separated by SDS-polyacrylamide gel electrophoresis. After Western blot transfer, proteasome C3 subunit protein was visualized using a mouse monoclonal antibody against the human C3 subunit. The apparent molecular mass of C3 subunit protein was ~26 kDa. The experiment was repeated three times with the same outcome. D, nuclei were isolated from vehicle-treated, control (Ctl) cells or cells treated with 50 nM dexamethasone (Dex) for 24 h according to Groudine et al. (22). Nuclear run-off assays were performed using isolated rat C3 proteasome or C3 control DNA fragments. Shown are the autoradiographic results from one set of assays performed from a single passage of cells; the experiment was repeated three times in duplicate with different cell passages with similar outcomes.

RESULTS

Catabolic Signals Increase Proteolysis and Proteasome C3 Subunit Expression in L6 Muscle Cells—Earlier, we found that protein degradation in BC3H1 myocytes was minimally increased by acidification or glucocorticoids, but with both signals, protein degradation was increased significantly (15). The weak response in BC3H1 cells may be related in part to their low level of glucocorticoid responsiveness compared with L6 skeletal muscle cells (15). In L6 cells, either extracellular acidification or dexamethasone (50 nM) significantly stimulated protein degradation (p < 0.02 versus control cells) but acidification plus glucocorticoid increased proteolysis the most (p < 0.05 versus other treatments; Fig. 1A). An equimolar concentration of the steroid receptor antagonist RU486 blocked the proteolytic response to dexamethasone (data not shown).

We studied the proteasome C3 subunit as a representative component of the ubiquitin-proteasome pathway because it is an α-subunit of the core proteolytic complex, and its transcription is increased in rat muscle by chronic uremia and acute diabetes (4, 6). We found that dexamethasone increased the
Fig. 2. Stimulation of C3 transcription by dexamethasone involves a NF-κB binding site. A, L6 cells were co-transfected with C3 promoter firefly luciferase and pTK-RL renilla luciferase control plasmids as indicated; luciferase activities in control and dexamethasone-treated cells (50 nM; 48 h) were measured. The fired luciferase activity (normalized for transfection efficiency) in control cells was determined and then used to calculate the percentage activity for each plate of dexamethasone-treated cells. Results are the mean ± S.E. (n ≥ 6 independent transfections/experiment) of the percentage of the activity in the respective control, untreated cells. Experiments with each plasmid were performed at least three times with the same outcome. B, the locations of elements homologous to a consensus NF-κB binding site in the human proteasome C3 subunit promoter regions from −450 to −200 (+1 is site of transcription initiation) are shown in bold; the orientation of each element is indicated by the arrow below the sequence.

amounts of both C3 subunit mRNA and protein −2-fold (Fig. 1, B and C). Using nuclear run-off assays, we determined that dexamethasone increased transcription of the C3 subunit gene nearly −2-fold but not the gene encoding GAPDH (Fig. 1D). Thus, glucocorticoids induce the expression of at least one component of the ubiquitin-proteasome pathway in L6 cells in a manner similar to conditions associated with increased corticosterone production and muscle wasting in rats (4–6).

Glucocorticoids Relieve Suppression of C3 Subunit Transcription by NF-κB—To determine how glucocorticoids stimulate transcription of the proteasome C3 subunit, we transfected L6 cells with a luciferase reporter plasmid containing the −460/+1 human C3 promoter fragment (+1 corresponds to the transcription start site) ligated to a firefly luciferase reporter gene (pC3−460). The transfected cells were incubated with dexamethasone (50 nM) for 48 h, which increased luciferase activity 2.9 ± 0.2-fold (p < 0.05) compared with untreated, transfected cells (Fig. 2A). This increase in luciferase activity was blocked when RU486 was added (118 ± 9% of the activity in control cells; p = not significant); RU486 alone did not change reporter enzyme activity (105 ± 11% of luciferase activity in untreated cells; p = not significant). To localize the glucocorticoid-responsive region of the C3 promoter, plasmids with shorter segments of the promoter sequence linked to the luciferase reporter gene were constructed. Shortening the promoter fragment to −400/+1 (pC3−400) did not prevent the induction of luciferase activity by dexamethasone, whereas trimming the promoter region to −256/+1 (pC3−256) abolished the dexamethasone response (Fig. 2A). The region of the C3 promoter between −400 and −256 does not contain a canonical glucocorticoid-response element but there are two elements similar to a consensus c-rel/NF-κB element: the downstream element (NF-κB1u) is in a forward orientation, whereas the upstream element (NF-κB2u) is in an inverted orientation (Fig. 2B). The functional importance of NF-κB1u in the dexamethasone response was investigated by replacing the base sequence of this site (−322/−313) with an unrelated sequence, while leaving the adjacent sequences unchanged in pC3−460 (pC3ΔNFκB1u). In cells transfected with pC3ΔNFκB1u, basal luciferase activity was not statistically different from the activity from cells transfected with an equal amount of pC3−460 (data not shown). Dexamethasone treatment did not increase luciferase activity in cells transfected with pC3ΔNFκB1u (Fig. 2A) indicating that the distal inverted c-rel/NF-κB sequence is necessary for the induction of C3 subunit transcription by glucocorticoids.

To address how NF-κB1u could be involved in the response to glucocorticoids, electrophoretic mobility shift assays were performed using a NF-κB1u DNA probe and protein extracts from nuclei isolated from control cells or cells treated with dexamethasone. Unexpectedly, we found an abundant nuclear protein(s) in extracts from untreated L6 muscle cells that complexed with the NF-κB1u probe (Fig. 3A). Treatment of L6 cells with dexamethasone reduced the amount of protein bound to NF-κB1u in a time-dependent fashion (Fig. 3A); the maximal decrease in protein binding occurred within 90 min of adding dexamethasone. Notably, the amount of the protein-NF-κB1u complex remained low when cells were treated for 24 h with a single dose of dexamethasone indicating that the effect of glucocorticoids was sustained. The interaction between the protein and NF-κB1u was specific because a 100-fold excess of either the unlabeled NF-κB1u probe or a probe corresponding to the immunoglobulin κ light chain gene NF-κB complex prevented the formation of the protein-DNA complex (Fig. 3B). Furthermore, anti-Rel A (p65) polyclonal antibodies induced a supershift in the mobility of the protein-NF-κB1u complex confirming that the protein complex contains a member of the NF-κB family (Fig. 4). Therefore, dexamethasone appears to increase C3 subunit expression by preventing activated NF-κB from binding to NF-κB1u.

To verify there is abundant activated NF-κB in untreated L6 cells, nuclear protein extracts from untreated cells were incubated with a DNA probe corresponding to the immunoglobulin κ light chain NF-κB binding site (Ig NF-κB) (28). A protein-Ig

Fig. 3. Dexamethasone reduces NF-κB binding to the C3 subunit NF-κB1u site. Nuclear protein extracts were prepared from control L6 cells or L6 cells treated with 50 nm dexamethasone, and EMSA was performed. A, cells were treated with dexamethasone for the time indicated. Equal amounts of nuclear proteins were incubated with a 32P-labeled NF-κB1u DNA probe before separation by polyacrylamide gel electrophoresis under non-denaturing conditions. A and B indicate the positions of the protein-DNA complex and unbound DNA probe, respectively. B, EMSA were performed with control cell nuclear protein extracts as in A (left lane) except that a 100-fold excess of competing DNA fragment containing NF-κB1u (middle lane) or the canonical immunoglobulin κ light chain NF-κB site (Ig NF-κB; right lane) was added to the binding reaction.
Fig. 4. p65/Rel A protein binds to NF-κB DNA probe complex. Nuclear protein extracts were prepared from untreated, control L6 cells, and EMSA was performed as described in Fig. 3A except that nuclear proteins were incubated with an antibody against the Rel A (p65) NF-κB subunit for 20 min before initiating the binding reaction with a labeled NF-κB probe. In the figure, B and U indicate the positions of the protein-DNA complex and unbound DNA probe, respectively. This experiment was performed twice and the outcome was the same. 

Signals That Activate NF-κB Also Suppress C3 Subunit Expression—If NF-κB acts to suppress C3 subunit transcription, then reducing the intracellular level of “activated” NF-κB should increase the basal C3 promoter activity (i.e., luciferase activity), and dexamethasone should induce even higher promoter activity. Conversely, raising activated NF-κB should suppress C3 subunit promoter activity, and the response to dexamethasone should be blunted. To address the first possibility, cells were cotransfected with pC3–460 and pCMV IxB(K21R/K22R), an expression plasmid encoding a dominant negative form of IxBα because of mutations that prevent conjugation of ubiquitin to it and hence, its degradation by the proteasome (25). Other cells were cotransfected with pC3–460 and pCMV IxB(S32A/S36A) to express another dominant negative form of IxBα in which serine is replaced by alanine in amino acid positions 32 and 36 to prevent phosphorylation of IxB by IκB kinase (25). Cells transfected with either type of dominant negative IxBα plasmid had a higher basal luciferase activity compared with cells transfected with pC3–460 alone (Fig. 6). When dexamethasone was added, luciferase activity from the cells cotransfected with pC3–460 and the mutant IxBα plasmids was higher than from the cells transfected with pC3–460 alone.

We also examined the influence of an inhibitor of NF-κB activation (100 μM pyrrolidine dithiocarbamate (28)) on C3 subunit expression and found that luciferase activity in L6 cells transfected with pC3–460 was 2.57 ± 0.55-fold higher (p < 0.05). Thus, blocking NF-κB activation stimulates C3 subunit transcription.

To determine if increasing the amount of activated NF-κB would suppress C3 subunit transcription, L6 cells were cotransfected with pC3–460 and plasmids encoding either p65 (pCMV-p65) or a p50/p65 chimeric protein composed of the DNA binding domain of p50 and the carboxyl-terminal transactivation domain of p65 of NF-κB (pCMV-p50/65 (24)). In both cases, the basal C3 promoter activity was below the activity in cells transfected with pC3–460 alone (Fig. 6); with dexamethasone, the increase in luciferase activity was sharply blunted compared with cells transfected with pC3–460 alone (Fig. 6). When the ratio of pCMV-p65 to pC3–460 during transfection was increased 5-fold, the basal luciferase activity was unchanged compared with cells transfected with the lower concentration of pCMV-p65, but dexamethasone no longer increased reporter activity (Fig. 6). Finally, we incubated L6 cells that had been transfected with pC3–460 with a mixture of cytokines (tumor necrosis factor-α, interferon-γ, and lipopolysaccharide) for 24 h to raise the level of endogenous activated NF-κB. This treatment increased the binding of NF-κB to the NF-κB probe, and basal luciferase activity was reduced by 48 ± 5% (p < 0.05 versus untreated, transfected cells; Fig. 7, A and B). As shown before, dexamethasone increased luciferase activity in cells transfected with pC3–460.

To determine if the endogenous proteasome C3 subunit gene also responds to cytokines, we incubated L6 cells with dexamethasone reduced the amount of this complex in a manner similar to the NF-κB probe (Fig. 5).
muscle cells were treated for the indicated times with dexamethasone (50 nM) and cytosolic and nuclear proteins were isolated. Proteins were separated by SDS-polyacrylamide gel electrophoresis, and after Western blot transfer, the NF-κB p65 subunit was visualized using a polyclonal antibody. A single 65-kDa protein was detected. The experiment was repeated three times with the same outcome. B, cytosolic proteins were isolated from control (0 min) L6 cells or cells incubated with dexamethasone for the indicated times. Proteins were separated by SDS-polyacrylamide gel electrophoresis and Western blots were prepared. The IκBα protein was visualized using a polyclonal antibody and a ~38-kDa protein was detected. The experiment was repeated three times with the same outcome.

**Stimulation of C3 Subunit Transcription by Acidification Does Not Require NF-κB**—A final issue we addressed was whether acidification and glucocorticoids act through similar mechanisms to stimulate the ubiquitin-proteasome system. The level of C3 subunit mRNA in control L6 muscle cells was compared with the level in cells that were acidified (pH 7.1) or cells incubated with dexamethasone at pH 7.1 or 7.4. Acidification plus dexamethasone increased C3 subunit mRNA more than either stimulus alone (data not shown). These results are similar to those we reported for ubiquitin and the proteasome C2 subunit mRNAs in C2C12 cells (15). We then determined if acidification alone stimulates the C3 promoter by acting at the NF-κB site; L6 cells were transfected with either pC3-460 or pC3NFκB(o) (containing an unrelated linker sequence instead of NF-κB(o)) and then incubating the cells at pH 7.4 or 7.1. In cells transfected with pC3-460, acidification increased luciferase activity from 46.9 ± 3 luminescence units in control cells (pH 7.4) to 114.3 ± 9.0 units (p < 0.05). Similar results were obtained when cells were transfected with pC3NFκB(o) (39.7 ± 3.3 at pH 7.4 versus 135.6 ± 14.6 at pH 7.1; p < 0.05).

**DISCUSSION**

Muscle atrophy in animal models of catabolic illnesses has been shown repeatedly to result from increased protein degradation because of activation of the ubiquitin-proteasome pathway (1). Concurrent with induction of this proteolytic pathway, there is increased transcription of its component genes in muscle suggesting that the two responses are linked. However, the complexity of animal models makes it difficult to identify specific signals initiating these responses. Our results in L6 muscle cells show that glucocorticoids increase the expression of the proteasome C3 subunit protein. Surprisingly, the mechanism involves antagonism of a transcriptional suppression imposed by constitutively active NF-κB. We also document that acidification, another stimulus that increases proteolysis in L6 cells, acts through a different mechanism because mutating the NF-κB(o) site that is required for dexamethasone-induced stimulation of the human C3 promoter did not prevent its induction by acidification.

There were several unexpected findings in our studies. First, a cis-acting element at position −322 ([i.e. NF-κB(o)]) in the
upstream promoter region of the human C3 proteasome confers glucocorticoid sensitivity, but this site is homologous to a classical NF-κB binding site rather than a glucocorticoid response element. Induction of C3 subunit expression by glucocorticoids is mediated by the glucocorticoid receptor because the receptor antagonist, RU486, blocked the transcriptional response. Secondly, our results indicate that NF-κB(u) acts as a negative transcriptional regulatory element. This finding was unanticipated, but it does provide an explanation for the report of Tamura et al. (23) that an unidentifed negative transcriptional element is located in the –439 to –256 region of the human C3 promoter region. Lastly, the protein that binds to NF-κB(u) in the C3 subunit gene is abundant in untreated L6 cells. This protein is a member of the NF-κB family (i.e., it was recognized by an anti-p65/Rel A antibody in mobility shift assays), and it appears to be a constitutively active suppressor of C3 subunit transcription in muscle cells. This was a surprise because NF-κB is typically an inducible transactivator that is inactive in the cytosol because of binding with the IκB inhibitor protein.

Are these studies with artificial C3 promoter-luciferase reporter chimeric genes relevant to the regulation of the endogenous C3 subunit gene in L6 cells? We believe they are because incubation of L6 cells with a cytokine mixture (to increase activated NF-κB (29, 30)): 1) raised the amount of NF-κB that binds to the NF-κB(u) probe and 2) decreased the amount of C3 subunit mRNA. Conversely, dexamethasone increased both the endogenous C3 subunit mRNA and the C3 promoter-driven luciferase activity. How do glucocorticoids decrease the binding of NF-κB to NF-κB(u) in the C3 proteasome subunit promoter? We found that dexamethasone increased the cytosolic level of IκBα, which appears to sequester NF-κB in the cytosol and prevent it from translocating into the nucleus and binding to NF-κB(u). Mechanisms that could raise the cytosolic level of IκBα include increased synthesis or reduced degradation of this protein. Others have reported that activated glucocorticoid receptors can block the interaction of NF-κB with its DNA target sequence by preventing NF-κB binding or antagonizing its transcriptional activation function through direct protein-protein interactions (31–33). Although we cannot exclude such actions by glucocorticoid receptors in L6 muscle cells, they seem unlikely because we did not detect protein-NF-κB(u) complexes exhibiting mobilities different from the NF-κB-NF-κB(u) complex in mobility shift assays performed with nuclear proteins from L6 cells treated with dexamethasone.

In summary, we have provided evidence for an important function of NF-κB in the transcriptional regulation of at least one component of the ubiquitin-proteasome proteolytic pathway in muscle. It is tempting to speculate that NF-κB regulates the transcription of other components of this pathway similarly because several of these genes have potential NF-κB-like binding sites in their promoter regions. These results also conclusively demonstrate that catabolic stimuli can increase the expression and amounts of components of the ubiquitin-proteasome proteolytic pathway. Finally, our results suggest novel mechanisms by which signals like acidification and glucocorticoids influence transcription of components of the ubiquitin-proteasome pathway and therefore, protein degradation in muscle.

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