Dexamethasone Represses Signaling through the Mammalian Target of Rapamycin in Muscle Cells by Enhancing Expression of REDD1*

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The mammalian target of rapamycin (mTOR), a critical modulator of cell growth, acts to integrate signals from hormones, nutrients, and growth-promoting stimuli to downstream effector mechanisms involved in the regulation of protein synthesis. Dexamethasone, a synthetic glucocorticoid that represses protein synthesis, acts to inhibit mTOR signaling as assessed by reduced phosphorylation of the downstream targets S6K1 and 4E-BP1. Dexamethasone has also been shown in one study to up-regulate the expression of REDD1 (also referred to RTP801, a novel stress-induced gene linked to repression of mTOR signaling) in lymphoid, but not nonlymphoid, cells. In contrast to the findings of that study, here we demonstrate that REDD1, but not REDD2, mRNA expression is dramatically induced following acute dexamethasone treatment both in rat skeletal muscle in vivo and in L6 myoblasts in culture. In L6 myoblasts, the effect of the drug on mTOR signaling is efficiently blunted in the presence of REDD1 RNA interference oligonucleotides. Moreover, the dexamethasone-induced assembly of the mTOR regulatory complex Tuberin-Hamartin is disrupted in L6 myoblasts following small interfering RNA-mediated repression of REDD1 expression. Finally, overexpression of Rheb, a downstream target of Tuberin function and a positive upstream effector of mTOR, reverses the effect of dexamethasone on phosphorylation of mTOR substrates. Overall, the data support the conclusion that REDD1 functions upstream of Tuberin and Rheb to down-regulate mTOR signaling in response to dexamethasone.

In contrast to the anabolic actions of growth-promoting hormones such as insulin and insulin-like growth factor 1, glucocorticoids act to repress protein synthesis in skeletal muscle of animals in vivo (1), in perfused hind limb preparations (2, 3), and in isolated muscle preparations (4, 5). In part, the repression of protein synthesis in response to glucocorticoids is a result of decreased signaling through a protein kinase referred to as the mammalian target of rapamycin (mTOR). 2 mTOR phosphorylates at least two proteins involved in the regulation of mRNA translation, the eukaryotic initiation factor (eIF)-4E-binding protein 1 (4E-BP1) and the ribosomal protein S6 kinase 1 (S6K1) (6). 4E-BP1 acts to repress mRNA translation by sequestering the mRNA cap-binding protein eIF4E into an inactive complex (7, 8). Phosphorylation of 4E-BP1 by mTOR initiates a series of phosphorylation events that ultimately result in the release of eIF4E from the inactive 4E-BP1-eIF4E complex, allowing it to bind to eIF4G to form the active eIF4F complex. Phosphorylation of S6K1 by mTOR generates a docking site for a second protein kinase, phosphoinositide-dependent protein kinase 1 (PDK1), allowing PDK1 to phosphorylate and activate S6K1 (9, 10). Subsequently, S6K1 phosphorylates ribosomal protein rpS6 (11), eIF4B (12, 13), and eukaryotic elongation factor (eEF2) kinase (14). Thus, repression of mTOR signaling results in a reduction in both the initiation and elongation phases of mRNA translation, resulting in a down-regulation of protein synthesis.

Previous studies have shown that the down-regulation of protein synthesis caused by dexamethasone administration to rats in vivo (15) or the addition of the drug to the culture medium of L6 myoblasts (16, 17) temporally correlates with reduced phosphorylation of the mTOR substrates 4E-BP1 and S6K1, suggesting that glucocorticoids might decrease protein synthesis through repression of mTOR. The dexamethasone-induced reduction in 4E-BP1 and S6K1 phosphorylation is attenuated both by inhibitors of glucocorticoid receptor function and inhibitors of transcription and translation (16). Moreover, the glucocorticoid receptor is both necessary and sufficient for the dexamethasone-induced dephosphorylation of S6K1 (18). Mutational analysis of the glucocorticoid receptor reveals that the DNA binding and transcriptional activation functions, but not the transcriptional repression function, of the receptor are required for S6K1 regulation (18). Overall, the available evidence strongly suggests that the repression of mTOR signaling in response to glucocorticoids is a result of enhanced transcription of a gene that encodes a repressor of 2 The abbreviations used are: mTOR, mammalian target of rapamycin; siRNA, short interfering RNA; eIF, eukaryotic initiation factor; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT, reverse transcription; S6K1, ribosomal protein S6 kinase 1; GAP, GTPase-activating protein; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonic acid; PKB, protein kinase B; ERK, extracellular-signal regulated kinase; AMPK, AMP-activated protein kinase.

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mTOR signaling. However, the identity of the putative mTOR repressor is unknown.

Recent studies have identified two novel repressors of mTOR signaling: proteins referred to as REDD1 and REDD2 (regulated in development and DNA damage responses; also known as RPT801 and RPT801L, respectively). REDD1 and REDD2 were originally identified as genes that are transcriptionally up-regulated in response to a variety of cellular stresses, including hypoxia (19–22) and exposure to arsenite (23), or by agents that cause DNA damage (24). More recent studies show that REDD1-induced repression of mTOR signaling requires Tuberin, the product of the TSC2 gene (19, 20, 25). Tuberin in a complex with Hamartin, the product of the TSC1 gene, functions as a GTPase-activating protein (GAP) toward a small G protein referred to as Rheb (Ras homologue enriched in brain), a positive upstream effector of mTOR (26). Binding of Rheb-GTP to mTOR enhances, whereas binding of Rheb-GDP inhibits, mTOR activity (27, 28). Thus, activation of Tuberin suppresses mTOR signaling by enhancing Rheb GTPase activity, resulting in an increase in the proportion of Rheb in an inhibitory complex with GDP (29–32). However, although it is clear that REDD1 requires Tuberin to repress mTOR signaling, the mechanism through which REDD1 might act to enhance Tuberin function is as yet undefined.

A recent study (33) reported that dexamethasone induces the expression of a gene referred to as dig2 (dexamethasone-induced gene-2). Sequencing of the dig2 mRNA revealed that it is identical to REDD1 (33), suggesting a possible link between induction of Dig2 expression and repression of signaling through mTOR. However, in that study, dexamethasone was found to induce REDD1 expression only in lymphoid cells and not in nonlymphoid cells, bringing into question the role of REDD1 in mediating the actions of glucocorticoids on mTOR signaling in other types of cells or tissues.

In the present study, the hypothesis that glucocorticoids repress mTOR signaling in skeletal muscle through induction of REDD1 and/or REDD2 was examined. In contrast to an earlier study (33) suggesting a lymphoid-specific up-regulation of REDD1 by dexamethasone, our results show that expression of REDD1 is enhanced by dexamethasone both in skeletal muscle in vivo and L6 myoblasts in culture. Moreover, REDD2 expression is repressed by dexamethasone in skeletal muscle and L6 myoblasts. Importantly, dexamethasone promotes the assembly of the active Tuberin-Hamartin complex, and complex assembly requires increased REDD1 expression. Finally, over-expression of Rheb attenuates the dexamethasone-induced repression of mTOR signaling. Overall, the results are consistent with a model wherein dexamethasone up-regulates REDD1 expression in muscle and REDD1 subsequently activates the Tuberin-Hamartin complex, resulting in repression of mTOR signaling.

EXPERIMENTAL PROCEDURES

Reagents—Dexamethasone (cell culture tested) and dexamethasone-sodium phosphate were purchased from Sigma. Anti-S6K1, anti-4E-BP1, and goat anti-rabbit and anti-mouse IgG horseradish peroxidase-conjugated antibodies were purchased from Bethyl Laboratories. Anti-Tuberin antibody was from Santa Cruz Biotechnology, and anti-Hamartin antibody was obtained from Cell Signaling Technologies. The antibody to REDD1 was purchased from ProteinTech Group (Chicago). Mouse monoclonal anti-eIF4E antibody was raised against recombinant human eIF4E by the method described earlier (34). BioMag goat anti-rabbit and goat anti-mouse polyclonal IgG beads were purchased from Qiagen. Enhanced chemiluminescence detection reagents were purchased from GE Healthcare. Predesigned REDD1 short interfering RNA oligonucleotides were purchased from Ambion (product numbers 193200, 193201, and 193202) as was the scrambled control oligonucleotide (product number 4635).

Animal Protocol—The animal experimental protocol used for the studies described herein was reviewed and approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine. Male Sprague-Dawley rats (200–300 g) were maintained on a 12 h light-12 h dark cycle with food and water provided ad libitum. Rats were injected intraperitoneally with dexamethasone-sodium phosphate (100 μg/100 mg of body wt) in sterile saline (0.15 M NaCl) (15). The control group received an equal volume of saline. Three hours later, half of the animals in each group were administered leucine (1.35 mg/kg of body weight) by oral gavage (35). Four hours after drug administration, rats were killed by decapitation. The left gastrocnemius and plantaris were rapidly excised as a unit and frozen between aluminum blocks precooled to the temperature of liquid nitrogen. The contralateral muscles were similarly excised, weighed, and homogenized in 7 volumes of ice-cold lysis buffer containing 20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM NaF, 100 mM KCl, 0.2 mM EDTA, 50 mM β-glycerophosphate, 1 mM dithiothreitol, 1 mM benzamidine, 0.5 mM sodium vanadate, and 0.1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged immediately at 1000 x g for 3 min at 4 °C, and the supernatant was collected for analysis.

Cell Culture, RNA Interference, and Transfection—L6 myoblasts were grown in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10% fetal bovine serum (Atlas) and 1% penicillin/streptomycin (Invitrogen) at 37 °C in a humidified atmosphere containing 5% CO2. Cells (1 x 106) were electroporated using a Nucleofector™ device (Ammax Biosystems) in the presence of 100 μl of Nucleofector Solution V and a mixture of three siRNAs (each at 1 μM) directed against REDD1. 48 h later, cells were deprived of serum for ~18 h and then treated with dexamethasone (1 μM) for 4 h prior to harvesting. Rat2 fibroblasts also were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10% heat-inactivated fetal bovine serum (Atlas). Transfections were performed using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer’s instructions.

Western Blot Analysis and Immunoprecipitation—The supernatants derived from muscle homogenates and cell lysates were combined with an equal volume of SDS sample buffer and boiled at 100 °C for 5 min. The samples were then subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted with primary antibody as indicated in the figure legends. Immunoreactive bands were visualized by enhanced chemiluminescence (ECL) using a GeneGnome HR bioimaging system (Syn-
Dexamethasone-induced REDD1 Expression Inhibits mTOR

gene). Immunoprecipitations were performed by incubating 1000 × g supernatants of muscle homogenate or cell lysate overnight with anti-eIF4E antibody or anti-Tuberin antibody followed by isolation of the immune complexes using BioMag IgG beads. The immunoprecipitates were washed 3 times with immunoprecipitation buffer (40 mM HEPES, pH 7.5, 120 mM NaCl, 1 mM EDTA, 10 mM sodium pyrophosphate, 10 mM β-glycerophosphate, 50 mM NaF, 0.3% CHAPS, 1 mM dithiothreitol, 1 mM benzamidine, 0.5 mM sodium vanadate, and 0.1 mM phenylmethylsulfonyl fluoride), and proteins were eluted by boiling in 1× SDS sample buffer. Samples were then subjected to Western blot analysis as described previously (36, 37).

Isolation of total RNA and Reverse Transcription—RNA was extracted from muscle and cells using Trizol reagent according to the manufacturer’s protocol (Invitrogen). RNA concentration and quality were assessed spectrophotometrically using a Beckman Coulter DU 640 (Fullerton, CA). RNA (1 μg) was reversed transcribed using 50 units of Superscript II RNase H − reverse transcriptase (Invitrogen) in a 21-μl reaction volume containing 0.5 μg/μl oligo(dT), 10 mM dNTP mix, 10× RT buffer (25 mM MgCl₂, 0.1 mM dithiothreitol), and RNaseOUT recombinant RNase inhibitor (Invitrogen). The mixture of RNA and oligo(dT) was heated at 65 °C for 5 min and then chilled on ice; then all other reagents were added, and RT-PCR was conducted at 42 °C for 50 min and 70 °C for 15 min. The reaction mixture was then diluted 1:8 before analysis by Real-time PCR as described below.

Real-time PCR Analysis of GAPDH, REDD1, and REDD2 mRNA—Quantitative Real-time RT-PCR was conducted using an Applied Biosystems Prism 7300 Real Time PCR system using a QuantiTech SYBR Green PCR kit (Qiagen) according to the manufacturer’s instructions. The primers used were as follows: GAPDH forward primer, 5′-GGGCTGCTTCTTCTTGTA-3′; reverse primer, 5′-TGACCATTGGCTGGTAGA-3′; REDD1 forward primer, 5′-TAGTGCCACCTTTTCACTTTG-3′; reverse primer, 5′-GTCAAGGGACTGGCTGTAACC-3′; REDD2 forward primer, 5′-CCAGCTCATGGACCTTCTC-3′; reverse primer, 5′-TCCTCAATGACTTGCTGTTCCC-3′. All PCR reactions were performed in 96-well optical plates (Applied Biosystems) containing each specific forward and reverse primer at 0.4 μM, 5 μl of diluted cDNA, and 12.5 μl of SYBR Green 1 in a total reaction volume of 25 μl. Each run included a buffer blank and no-template control to test for contamination of assay reagents. Gene expression levels were calculated and normalized to GAPDH mRNA expression.

Statistical Analysis—The data are expressed as mean ± S.E. One-way analysis of variance and Student’s t test were used to compare differences among groups. p < 0.05 was considered statistically significant.

RESULTS

A previous study (38) showed that glucocorticoids repress mTOR signaling in skeletal muscle in rats and that leucine administration to dexamethasone-treated rats reverses the repression. The present study confirmed the results of the earlier study and showed that 4 h after dexamethasone administration, phosphorylation of S6K1 and 4E-BP1 was reduced in skeletal muscle (data not shown). To assess whether either dexamethasone or leucine administration alters the expression of REDD1 or REDD2, the amounts of the respective mRNAs in rat skeletal muscle RNA were measured by RT-PCR and expressed relative to the amount of GAPDH mRNA, an mRNA that did not change in response to either dexamethasone or leucine administration. As shown in Fig. 1A, 4 h after dexamethasone administration, the expression of REDD1 mRNA was increased more than 8-fold above the control value. In contrast, REDD2 mRNA expression was reduced in muscle from dexamethasone-treated rats compared with controls (Fig. 1B). Leucine, administered to either control or dexamethasone-treated rats had no significant effect on the expression of either form of mRNA.

To confirm that the dexamethasone-induced changes in REDD1 mRNA abundance would result in altered REDD1 protein expression, the effect of the hormone on REDD1 expression was assessed by protein immunoblot analysis. It was found that REDD1 protein content was almost undetectable in muscle from control rats but was dramatically increased in muscle of rats treated with dexamethasone (Fig. 2A). Thus, the increase in REDD1 mRNA abundance is associated with a corresponding increase in protein expression.

To further explore the regulation of REDD1 and REDD2 mRNA expression and mTOR signaling by dexamethasone, the effect of the drug in L6 myoblasts was examined. As shown in Fig. 3, mTOR signaling was repressed in L6 myoblasts 4 h after
Dexamethasone administration, as assessed by a shift of both S6K1 (Fig. 3A) and 4E-BP1 (Fig. 3B) into less phosphorylated, i.e. more rapidly migrating, forms. As observed in skeletal muscle from dexamethasone-treated rats, dexamethasone enhanced the expression of REDD1 mRNA (Fig. 3C) and simultaneously repressed REDD2 mRNA expression in L6 myoblasts (Fig. 3D). Moreover, dexamethasone treatment resulted in a 5-fold increase in REDD1 protein content compared with controls (Fig. 2B).

To assess whether or not the enhanced expression of REDD1 mRNA was necessary for repression of mTOR signaling, siRNAs directed against the rat REDD1 mRNA were used to reduce its expression in L6 myoblasts. As shown in Fig. 4A, administration of REDD1 siRNA caused a reduction in REDD1 mRNA expression to ~50% of the value observed in cells that had been administered a control siRNA. Moreover, administra-

**FIGURE 2.** Dexamethasone enhances REDD1 protein expression in skeletal muscle and L6 myoblasts. A, fasted rats (control) were injected with dexamethasone, and 1,000 × g supernatants were subjected to protein immunoblot analysis as described under “Experimental Procedures.” The inset depicts the results of a typical blot for control (C) and dexamethasone-treated (D) rats. The results represent the mean ± S.E. of five animals for each group. B, L6 myoblasts were deprived of serum for 18 h and then exposed to 1 μM dexamethasone or vehicle for 4 h prior to harvest as described under “Experimental Procedures.” REDD1 protein content in cell lysates was assessed by protein immunoblot analysis. The inset depicts the results of a typical blot for control and dexamethasone-treated cells. The results represent the mean ± S.E. of six dishes of cells/condition. *, p < 0.001 versus control.

**FIGURE 3.** Dexamethasone down-regulates mTOR signaling and REDD2 expression but enhances REDD1 expression in L6 myoblasts. L6 myoblasts were exposed to dexamethasone (dex) or vehicle control (con) as described in the legend to Fig. 2B. Cell homogenates were analyzed for the phosphorylation state of S6K1 (A) and 4E-BP1 (B) by changes in migration during SDS-PAGE. The α, β, γ, and δ forms of the proteins are denoted to the right of the respective blots. The results are representative of four studies performed. Within each study, three dishes of cells were analyzed individually. Representative blots are shown. RNA was isolated from L6 myoblasts and analyzed for the amount of REDD1 (C), REDD2 (D), and GAPDH mRNAs (not shown) present in the sample by quantitative RT-PCR as described under “Experimental Procedures.”

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through which mTOR signaling is regulated involves the Tuberin-Hamartin complex. As shown in Fig. 4D, dexamethasone enhanced the amount of Hamartin associated with Tuberin in L6 myoblasts transfected with control siRNA. However, the drug had no effect on assembly of the Tuberin-Hamartin complex in cells transfected with REDD1 siRNA, suggesting that REDD1 may function upstream of Tuberin-Hamartin in regulating mTOR signaling.

To further assess a possible role for the Tuberin-Hamartin complex in REDD1-mediated repression of mTOR signaling, the effect of exogenous expression of Rheb on the dexamethasone-induced repression of mTOR signaling was examined. For these studies, Rat2 cells were used because Rheb was only poorly expressed in transfected L6 myoblasts (data not shown). As shown in Fig. 5A, Rheb expression was increased by at least an order of magnitude in Rat2 cells transfected with the FLAG-Rheb plasmid compared with cells transfected with the control plasmid. As observed in L6 myoblasts, dexamethasone caused a decrease in phosphorylation of both S6K1 (Fig. 5B) and 4E-BP1 (Fig. 5C) as assessed by decreased migration during electrophoresis. In contrast, exogenous expression of Rheb enhanced both S6K1 and 4E-BP1 phosphorylation in the absence of dexamethasone. Importantly, in cells overexpressing Rheb, the effect of dexamethasone on S6K1 and 4E-BP1 phosphorylation was severely attenuated.

**DISCUSSION**

The results of the present study confirmed earlier reports that glucocorticoids repress mTOR signaling in skeletal muscle.
Dexamethasone-induced REDD1 Expression Inhibits mTOR

(15, 38) and L6 myoblasts (16). Thus, in both gastrocnemius muscle and L6 myoblasts, dexamethasone promotes dephosphorylation of the mTOR substrates S6K1 and 4E-BP1. The present study extended the findings from previous studies to show that REDD1, but not REDD2, mRNA expression is enhanced in both skeletal muscle and L6 myoblasts in response to dexamethasone administration. In a previous study (33), dexamethasone was shown to induce REDD1 expression in lymphocytes but not in the nonlymphoid cells that were examined. Although mTOR signaling was not examined in the earlier study, the time course for induction of REDD1 mRNA by dexamethasone in lymphoma cells (33) parallels that for the repression of mTOR signaling in L6 myoblasts (16). Moreover, both dexamethasone-induced expression of REDD1 mRNA in lymphocytes (33) and repression of mTOR signaling in L6 myoblasts (18) are blocked by RU486, a glucocorticoid agonist that competes with dexamethasone for binding to the glucocorticoid receptor. Overall, the available evidence indirectly suggests that dexamethasone might act to repress mTOR signaling through a glucocorticoid receptor-mediated increase in REDD1 expression. That suggestion is supported by the results of the present study showing that dexamethasone induces REDD1 expression not only in lymphoid cells, as reported previously (33), but also in skeletal muscle in vivo as well as in L6 myoblasts in culture.

A number of recent studies have suggested a possible link between induction of REDD1 expression and repression of signaling through mTOR. Studies in both Drosophila (20) and mammalian cells (39) show that exogenous overexpression of either REDD1 or REDD2 represses mTOR signaling, whereas decreased expression of either protein increases signaling through mTOR. Moreover, in mouse embryo fibroblasts lacking REDD1, conditions that normally promote expression of the protein have little effect on the phosphorylation state of S6K1 or 4E-BP1 (25).

One mechanism through which mTOR is regulated involves its association with Rheb. Rheb binds to mTOR near the amino-terminal end of its catalytic domain (27). When it is associated with GTP, Rheb acts to enhance mTOR activity, but when it is associated with GDP, it is a repressor. The finding in the present study, that overexpression of Rheb in L6 myoblasts attenuates the down-regulation of mTOR signaling caused by dexamethasone, suggests that the drug may act to repress mTOR signaling by enhancing the proportion of Rheb present in the GDP-bound form. Such a suggestion is also supported by the observation that overexpression of Rheb in mouse embryo fibroblasts reverses the effect of exogenous expression of REDD1 on mTOR signaling (25).

Further evidence supporting the suggestion that dexamethasone may act to increase the amount of Rheb in the GDP-bound form is the novel observation that dexamethasone enhances the assembly of the Tuberin-Hamartin complex (Fig. 4). Moreover, REDD1 knockdown using siRNA prevented dexamethasone-induced assembly of the complex, suggesting that REDD1 acts to repress mTOR by signaling to Tuberin and Hamartin. Although Tuberin by itself exhibits GAP activity toward Rheb in in vitro assays, co-expression of both Tuberin and Hamartin is required for effective repression of mTOR signaling in cells in culture (40). Moreover, mutations in Tuberin that interfere with its binding to Hamartin repress the growth inhibitory effects of the protein in COS1 cells (41). That REDD1 functions through the Tuberin-Hamartin complex to modulate mTOR function is further supported by recent studies showing that REDD1 signaling to mTOR requires Tuberin. For example, in cells in which Tuberin expression is reduced using siRNA, induction of REDD1 expression has little or no effect on S6K1 phosphorylation (19, 42). Moreover, in contrast to wild type cells, exogenous expression of REDD1 in mouse embryo fibroblasts lacking Tuberin has no effect on S6K1 phosphorylation (25). However, to our knowledge, the effect of exogenous expression of REDD1 on assembly of the Tuberin-Hamartin complex has not been examined previously.

Although the mechanism through which REDD1 might regulate Tuberin function is unknown, one possibility involves changes in Tuberin phosphorylation. In this regard, Tuberin is regulated through phosphorylation by a number of protein kinases. For example, phosphorylation of Tuberin on multiple residues by protein kinase B (PKB; also known as Akt) is associated with inhibition of its GAP activity toward Rheb, resulting in enhanced signaling through mTOR (reviewed in Ref. 43). However, in mammalian cells, overexpression of REDD1 has no effect on PKB phosphorylation (39), and in Drosophila REDD1 functions downstream of PKB (20) suggesting that REDD1 does not regulate Tuberin through a PKB-dependent process. Tuberin function is also regulated through phosphorylation by the extracellular-signal regulated kinase (ERK) (44), but the finding that dexamethasone does not activate ERK in L6 myoblasts (15) suggests that ERK is not downstream of REDD1. The AMP-activated protein kinase (AMPK) also phosphorylates Tuberin, and in contrast to phosphorylation by PKB or ERK, phosphorylation by AMPK enhances Tuberin activity, resulting in repressed mTOR signaling (45). However, although REDD1 does not require AMPK to repress mTOR signaling (19, 25), AMPK requires REDD1 to modulate mTOR activity (25). Overall, the available evidence suggests that REDD1 modulates Tuberin-Hamartin assembly through an as yet undiscovered mechanism.

Of relevance to the mechanism through which REDD1 might modulate Tuberin function is the observation that oral administration of leucine to fasted rats has no effect on either basal or dexamethasone-induced REDD1 expression. In contrast, the magnitude of the leucine-induced increase in 4E-BP1 and S6K1 phosphorylation is unaffected by dexamethasone (38), suggesting that leucine acts downstream of REDD1 to activate mTOR. Amino acids have little (28, 46) or no effect (47) on the amount of GTP bound to Rheb. Moreover, in cells lacking Tuberin, amino acid deprivation represses mTOR signaling, and the readdition of amino acids to deprived cells restores phosphorylation of 4E-BP1 and S6K1 (28, 46), suggesting that amino acid regulation of mTOR can occur through a Tuberin-independent mechanism. Although the putative Tuberin-independent signaling pathway regulated by amino acids has not been delineated, a recent study suggests that amino acids promote the association of Rheb with mTOR and thereby enhance mTOR protein kinase activity (27). Therefore, in part, amino acids may overcome the repressive effects of glucocorticoids on
Dexamethasone-induced REDD1 Expression Inhibits mTOR

mTOR signaling by stimulating the binding of Rheb to mTOR. A second possibility is that amino acids might promote the activation of a guanine nucleotide exchange protein (GEF) for Rheb. Although a Rheb GEF has yet to be identified, activation of such a protein might overwhelm the repressive effect of REDD1-mediated Tuberin activation and thereby enhance mTOR signaling. Such an idea will need to be addressed in future studies.

In conclusion, the present work demonstrates a pivotal role for REDD1 in down-regulating mTOR function in response to dexamethasone treatment. The results of the present study, in combination with previously published reports, support a model wherein dexamethasone binds to the glucocorticoid receptor, thereby enhancing REDD1 gene transcription. Through an as yet unidentified mechanism, REDD1 promotes the association of Hamartin with Tuberin (41) resulting in enhanced GAP activity toward Rheb. The resulting increase in Rheb complexed with GDP represses mTOR function, leading to decreased phosphorylation of both S6K1 and 4E-BP1 and subsequent alterations in mRNA translation and protein synthesis.

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