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Evidence for Down-Regulation of Phosphoinositide 3-Kinase/Akt/Mammalian Target of Rapamycin (PI3K/Akt/mTOR)-Dependent Translation Regulatory Signaling Pathways in Ames Dwarf Mice

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How growth hormone (GH) stimulates protein synthesis is unknown. Phosphoinositide 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathways balance anabolic and catabolic activities in response to nutrients and growth factor signaling. As a test of GH signaling, immunoassays of two downstream translation regulatory proteins were compared in ad libitum-fed 2-month-old normal and Ames (Prop1*df*) dwarf mice. Phosphorylation of the p70 and p85 isoforms of S6 kinase 1 in liver and the p70 isoform in gastrocnemius muscle were significantly decreased in dwarfs. Messenger RNA (mRNA) Cap-binding demonstrated significantly higher levels of translation repressor 4E-BP1/eukaryotic initiation factor 4E (eIF4E) (coprecipitates) from dwarf livers, but not muscle. Consistent with these binding data, significantly less phosphorylation of 4E-BP1 was documented in dwarf liver. These data suggest a link between GH signaling and translation control in a model of extended longevity.

GROWTH hormone (GH) and growth hormone receptor (GHR) are members of a large family of cytokine peptides (1,2) and receptors (3,4) vital for somatic growth. The complex hormonal regulation of somatic growth is not fully understood (5–7). In addition to growth, GH has recently been implicated in immune function (8), cognition (9), and longevity (10,11). GH therapy in children of short stature (12) and in GH-deficient adults (13,14) is prevalent, although negative side effects such as promotion of insulin resistance and associated complications are recognized (15–18). Intertwined with the stimulatory effects on growth are GH effects on metabolism, such as decreased adiposity and increased lean body mass (19). Included among GH effects on metabolism is stimulation of protein synthesis, although the signaling pathways are unknown (20).

Ames (Prop1*df*) and Snell (Pit-1*dw*) dwarf mice carrying single gene mutations resulting in hypopituitarism and combined pituitary hormone deficiencies (in GH, thyroid-stimulating hormone, and prolactin) have been valuable models in the study of the hormonal regulation of growth (21). Consistent with the classic and revised somatomedin hypotheses (5–7), the deficiency of GH in Ames dwarfs accompanies reductions in circulating insulin-like growth factor-1 (IGF-1) produced by the liver (reviewed in [22]), suggesting that the reduction of this endocrine growth factor contributes to their small size. However, liver-specific knockout of IGF-1 in mice did not lead to reduction in somatic growth (23,24), indicating that extrahepatic IGF-1 and GH are sufficient to support normal growth, perhaps through autocrine and/or paracrine stimulation (7,25). Gigantism in mice deficient for suppressor of cytokine signaling-2 (SOCS-2) gene expression, which is GH regulated, supports an important role for the autocrine actions of GH and IGF-1 in growth (26). Thus, reduced GH and/or IGF-1 autocrine and/or paracrine signaling may be a primary contribution to the small size of GH-deficient dwarfs. Reduced muscle fiber (cell) sizes, but not fiber number, in Ames dwarf mice was reported (27). It is interesting that GH treatment of Snell dwarfs was associated with increased centrilobular hepatocyte cell size, an effect that was abrogated by cotreatment with IGF-II, and to a lesser extent by IGF-1 (28). Results from studies with yeast, *Drosophila*, and mice (29,30) suggest that cell growth (increase in mass) and proliferation result from the convergence of at least two sets of cues: signaling from the PI3K and Akt kinases and the presence of sufficient nutrients/energy as sensed by the anabolic and/or catabolic balancer kinase, mammalian target of rapamycin (mTOR).

The phosphoinositide 3-kinase (PI3K) pathways affect cell growth, proliferation, survival, and motility (31). The lipid products of PI3K provide localized membrane anchors for the assembly of various signaling proteins, which have domains that bind to D3 phosphorylated phosphoinositides. Among these are Akt, a serine threonine kinase also known as protein kinase B (PKB), and phosphoinositide-dependent kinase 1 (PDK1). One of the downstream effectors of the PI3K and Akt pathways important in metabolic regulation is S6 kinase (two homologues in mammals), which is suggested to control ribosome biogenesis by phosphorylating S6 ribosomal protein, and possibly translation initiation by phosphorylating eukaryotic initiation factor 4B (eIF4B) (32).

Other downstream effectors important in the regulation of Cap-dependent initiation of translation are a family of

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repressors called 4E-BPs (also called PHAS, [33,34]). By acting as ''molecular mimics'' of the eIF4E-binding site in eIF4G (35), these repressors regulate the assembly of the mammalian eIF4F trimeric Cap-binding initiation complex (eIF4E, eIF4G, and eIF4A) by competing for eIF4E-eIF4G binding (36). Hyperphosphorylation of 4E-BP1 abrogates its interaction with eIF4E (33,34), thereby de-repressing Cap-dependent translation.

S6K activation and phosphorylation of 4E-BP1 are both dependent on PI3K and mTOR signaling (37,38). mTOR (also known as FKBP12-rapamycin complex-associated protein 1, FRAP1, in humans; rapamycin and FKBP12 target-1, RAFT1 in rats; sirolimus effector protein, SEP; and RAPT) is in a family of phosphotidylinositol (PI) kinase-related kinases. mTOR is postulated to balance anabolic and catabolic cellular activities to nutrient levels and growth factor signaling (reviewed in [37–41]). S6 kinase 1 (S6K1) and 4E-BP1 both function to regulate mammalian cell growth (size) (29,30).

In this study, we investigated the status of S6K1 and 4E-BP1 in liver and muscle from Ames dwarf mice and normal size littermates. Compared to normal size littermates, we obtained immunoassay evidence for significantly less phosphorylation of S6K1 in both liver and muscle. Less phosphorylation of 4E-BP1, in concert with increased mRNA Cap-binding 4E-BP1-eIF4E coprecipitates were documented. Decreased eIF4G-eIF4E coprecipitates in dwarf liver, but not muscle, were also recorded.

Experimental Animals

This study involved 10 homozygous Ames dwarf female mice (*df*/*df*) weighing 8–12 g each and 10 of their normal size female siblings (*df*/+ or +/+) weighing 20–30 g each. The mice were fed standard laboratory mouse pellets ad libitum, and ranged from 8 to 10 weeks of age at the time they were killed. The procedures and experiments involving use of mice were approved by the Institutional Animal Care and Use Committee and are consistent with the National Institutes of Health Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Education, the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (National Academy Press, Washington, D.C.).

Methods

Lysate Preparation

Tissues were dissected and immediately frozen in liquid nitrogen and stored at −75°C. Tissue chunks were powdered under liquid nitrogen, from which protein extracts for immunoblots were prepared (42). For Cap-binding assays, the frozen tissues were powdered in liquid nitrogen, and proteins were freeze-thaw extracted in K150T buffer (150 mM KCl, 50 mM Tris-HCl (pH 7.4), 0.125% Na deoxycholate, 0.375% Triton X-100, 0.15% Nonidet P-40, 4 mM EDTA, 50 mM NaF). Proteins were quantified using Bio-Rad DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA).

Immunoblots

Extracts prepared from liver and gastrocnemius muscle from *df*/*df* mice ($n = 10$) and normal size littermates ($n = 10$) were immunoassayed to determine the phosphorylation status of PI3K, Atk and mTOR downstream effectors. Studies have placed S6K1 downstream of mTOR and PI3K/Atk kinases (32,37). Alternate translation initiation generates two isoforms of S6K1: p70, which is observed primarily in the cytoplasm, and p85, which, by virtue of an amino-terminal nuclear localization sequence, is localized in the nucleus (43). S6K1 is regulated by multisite phosphorylation in both isoforms, including rapamycin-sensitive Thr389, Thr421, and Ser424 (32). In the following analyses, the status of these phosphorylation sites was assayed using phosphorylation state-dependent and -independent antibodies on the same immunoblots. In each case, the signal intensity obtained with the phosphorylation state-dependent antibody was normalized to that obtained with the phosphorylation state-independent antibody.

After fractionation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), proteins were transferred to nitrocellulose membranes (Current Protocols in Molecular Biology, www.mrw2.interscience.wiley.com), which were then blocked in 5% nonfat dry milk in Tris-buffered saline–Tween 20. The membranes were incubated in primary antibody solution containing one from the following suppliers: (a) Cell Signaling Technology (#9204 that specifically detects Thr421 and Ser424-phosphorylated S6K1, #9206 1A5 monoclonal antibody or #9205 rabbit polyclonal antibodies that specifically detect Thr389-phosphorylated S6K1, #9742 that detects eIF4E, #9458 that specifically detects Thr46-phosphorylated 4E-BP1, #2211 that detects Ser235 and Ser236-phosphorylated S6 ribosomal protein, or #2212 that detects S6 ribosomal protein); (b) Santa Cruz Biotechnology (sc-6936 that specifically detects 4E-BP1 or sc-230 that detects S6K1); and (c) N. Sonenberg, Department of Biochemistry, McGill University (rabbit polyclonal antibodies for eIF4G1 and eIF4G2). After incubation overnight at 4°C, the primary antibodies were detected by immunoglobulin G-horseradish peroxidase conjugate and SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL). After varied time exposures to X-ray film, the blots were stripped using Restore reagent (Pierce Biotechnology), checked for removal of the substrate and primary antibody, and re-immunoassayed as indicated. Immunoassaying some of the initial blots with α-rat Pit-1 antibody served as a negative control (data not shown).

Cap-Binding Assays

Messenger RNA Cap coprecipitation assays for 4E-BP1were done using varying amounts of K150T (150 mM KCl, 50 mM Tris-HCl (pH 7.4), 0.125% Na deoxycholate, 0.375% Triton X-100, 0.15% Nonidet P-40, 4 mM EDTA, and 50 mM NaF) tissue lysates and 7-methyl guanosine triphosphate (GTP)-Sepharose (Amersham Biosciences, Little Chalfont, UK). Lysates, 50–100 μl, were first cleared by a 1-hour incubation in 750 μl of LCB binding buffer (20 mM Tris-HCl (pH 7.4), 0.2 mM EDTA, 100 mM KCl, 7 mM β-mercaptoethanol, 1X Complete Protease Inhibitors, Roche), and 100 μl of deactivated CnBr-Sepharose. After removal of the deactivated CnBr-Sepharose by gentle centrifugation, 100 μl of 7-methyl GTP-Sepharose was added

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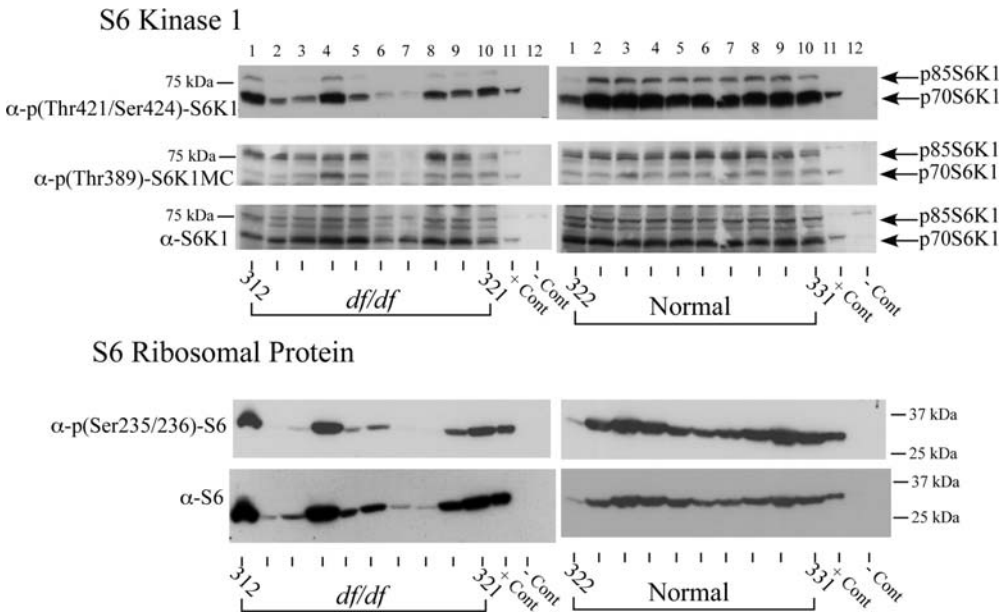

Figure 1. Phosphorylation of liver S6 kinase 1 and S6 ribosomal subunit protein in Ames dwarf (*df/df,* mice 312-321, lanes 1–10, left) and normal size littermates (mice 322-331, lanes 1–10, right). *Top panels:* Lysates were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoassayed with antibodies to S6 kinase 1, which are indicated on the left. The two known isoforms of S6K1 are indicated on the right. *Bottom panels:* The same lysates as above were immunoassayed with antibodies to S6 ribosomal protein, which are indicated on the left. Phosphorylation controls, NIH3T3 cells treated with (+Cont) and without (–Cont) serum, are in lanes 11 and 12, respectively.

to the binding reaction, and gently mixed for 2 hours at 4°C. After washing, the resin was washed three times in LCB binding buffer, mixed with an equal volume of 2X SDS–PAGE loading buffer, and boiled for 3 minutes; 28 μl was loaded onto SDS–PAGE gels, which were immunoblotted for 4E-BP1 and eIF4E, as described above.

Blot Analyses

Band intensities were obtained by scanning developed X-ray films (Personal Densitometer, Molecular Dynamics, now Amersham Biosciences) and quantified using OptiQuant (PerkinElmer Life and Analytical Sciences, Boston, MA). Statistical tests (*t* tests, Mann–Whitney nonparametric tests, and Wilcoxon signed ranked comparison) were performed using GraphPad Prism (version 3.00, GraphPad Software, San Diego, CA, www.graphpad.com). At least three repetitions of each Western blot experiment in Figures 1 and 2 were performed, with similar results.

Results

An analysis of *df/df* liver extracts showed 3.0- and 2.3-fold less phosphorylation at Thr421/Ser424 sites in the p70 and p85 isoforms, respectively, relative to the total of each S6K1 isoform (Figure 1 and Table 1, $p = .0006$ and $p = .0012$, unpaired *t* tests, respectively) to those of normal size littermates. Quantification of p(Thr389)-S6K1 indicated 3.2-fold less phosphorylation of this residue in the p70 isoform only (Table 1, $p = .036$, unpaired *t* test) in dwarf liver compared to that of normal size littermates. The data indicate no differences in the p85 isoform phosphorylated on Thr389 in the liver extracts.

An analysis of muscle extracts showed 2.2-fold less phosphorylation at the Thr421/Ser424 site in the p70 isoform in lysates from dwarf mice compared to normal littermates (Figure 2 and Table 1, $p = .035$, Mann–Whitney nonparametric test). Phosphorylation at the Thr421/Ser424 site in the p85 isoform was not detected in muscle extracts of normal or dwarfs, whereas both isoforms were readily detected with phosphorylation state-independent antibodies (Figure 2). There were no differences in the phosphorylation of the Thr389 site in the p85 isoform in muscle lysates from dwarf mice compared to normal littermates (Figure 2). Phosphorylation of Thr389 in the p70S6K1 isoform was not detected in the muscle proteins of either dwarf or normal mice (Figure 2). In liver, these differences in isoform phosphorylation were not observed (Figure 1). Both the liver and muscle experiments were repeated three times using separate aliquots of extract and blots, and similar results were obtained.

One of the principal substrates of S6K1 is the sixth ribosomal subunit (S6). The phosphorylation of S6 has been suggested to enhance translation of 5′ terminal oligopyrimidine (TOP) mRNAs (32), which encode products for ribosome biogenesis. Ser235/236 is one of the principal sites targeted for S6 phosphorylation, and was separately assayed. There were no differences in the ratios of Ser235/236-phosphorylated S6 to total protein in the immunoblots of liver of dwarf and normal size mice and of muscle extracts compared to the corresponding tissues of normal mice (Figures 1 and 2).

To functionally compare 4E-BP1 in dwarf and normal liver, an mRNA Cap affinity resin, 7-methyl-GTP-Sepharose, was used to assay 4E-BP1 that coprecipitates with the Cap-binding protein, eIF4E. Extracts were incubated with the resin, and 4E-BP1 and eIF4E associated with the beads

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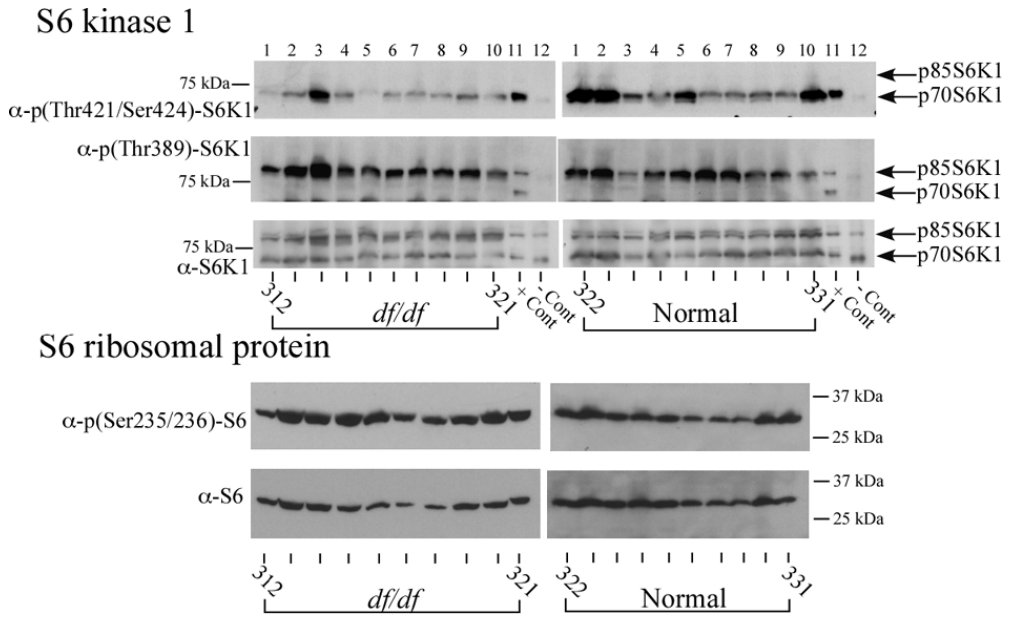

Figure 2. Phosphorylation of muscle S6 kinase 1 and S6 ribosomal subunit protein in Ames dwarf (*df/df,* mice 312-321, lanes 1–10, left) and normal size littermates (mice 322-331, right). *Top panels:* Lysates prepared from gastrocnemius muscle were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoassayed with the antibodies indicated on the left. The two known isoforms of S6K1 are indicated on the right. *Lower panels:* The same lysates as above were immunoassayed with the antibodies indicated on the right. Phosphorylation controls, NIH3T3 cells treated with (+Cont) and without serum (–Cont), are lanes 11 and 12, respectively.

were detected by immunoassays of Western blots. The band intensities of 4E-BP1 were normalized to that of the eIF4E for each experiment. Shown in Figure 3 are data from 8 representative pairs of liver (3A) and muscle (3B) samples from dwarf and normal mice that illustrate the ranges observed in these assays. Indicated below each experiment is the ratio of dwarf to normal 4E-BP1 binding. A comparison of 10 pairs of binding assays indicated that, on average, there is a 7.3-fold greater coprecipitation of 4E-BP1 in lysates prepared from dwarf liver compared with those from normal liver ($p = .001$, Wilcoxon signed ranked comparison). In addition to pair-wise comparisons, a group analysis of the liver data showed an average 3.5-fold increase in binding in dwarf compared to normal size littermates ($p = .025$, unpaired t test). When the gels resolved the three mobility forms of 4E-BP1 (44), the dwarf lysates, consistent with binding data, showed less of the highest phosphorylated and slowest migrating γ form (e.g., see animal number 45318 compared to 45329, and 45313 compared to 4536 in Figure 3A). Similar analyses of 10 binding assays of muscle lysates showed no significant differences between dwarf and normal size littermates.

Prevailing models of 4E-BP1 posit that it functions as a molecular mimic that competes with eIF4G for eIF4E binding (35). This model predicts that eIF4G binding should be opposite to that of 4E-BP1 in the Cap binding assays. Figure 4 shows the levels detected by rabbit antibodies to eIF4G1 and eIF4G2 in a binding reaction of liver proteins from dwarf (45318) and normal (45329) mice. In this experiment comparing 1 pair of mice, the decrease in eIF4G1/2 binding in dwarf is threefold, consistent with increased 4E-BP1 binding (Figure 3).

Hyperphosphorylation of 4E-BP1 in response to growth factor signaling is postulated to inhibit its binding to eIF4E, thereby de-repressing translation initiation. According to this model and data in Figures 3 and 4, phosphorylation of 4E-BP1 should be decreased in dwarf mice compared to normal mice. Figure 5 shows immunoblot data developed with phosphorylation state-dependent (Thr46) and -independent 4E-BP1 antibodies. Consistent with the binding data, dwarf liver extracts showed more than sevenfold less Thr46 phosphorylation compared to that in normal liver extracts ($p = .0009$, Mann–Whitney test). Figures 3 and 5 show that, consistent with the phosphorylation data, the slower mobility forms of 4E-BP1 (more highly phosphorylated) are also reduced in the dwarf extracts.

Discussion

As early as 1965, Korner (45) argued that, irrespective of the types of proteins, the increase in the rate of protein synthesis in the liver by GH is regulated at the cytoplasmic

Table 1. Summary of Significant Differences in S6 Kinase 1 Phosphorylation Between Dwarf and Normal-Size Mice

	Liver		Muscle	
Phosphorylation Site	p70	p85	p70	p85
Thr421/Ser424	*	†	‡	ND
Thr389	§	(–)	ND	(–)

Notes: *p value = .0006, unpaired t test.
†p value = .0012, unpaired t test.
‡p value = .035, unpaired t test.
§p value = .0362, unpaired t test.
ND = not detected; (–) = no difference.

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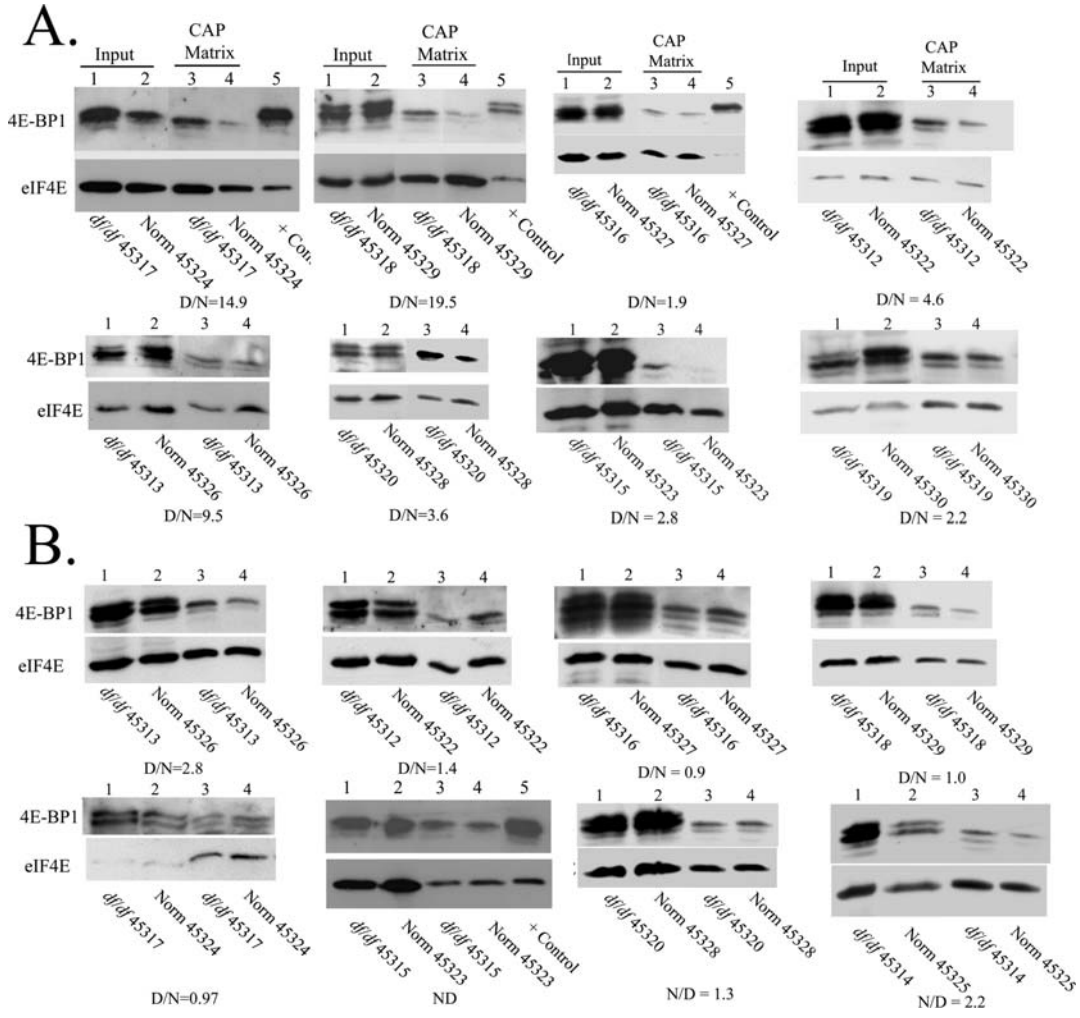

Figure 3. Increased 4E-BP1/eIF4E repressor complexes in Ames dwarf liver. Shown are representative m7-guanosine triphosphate (GTP) Cap-binding coprecipitation assays for dwarf and normal liver (**A**) and muscle (**B**) extracts. Input (lanes 1 and 2) is 12.5 μl of the binding lysates used in each experiment. Cap Matrix (lanes 3 and 4) is 28 μl of the matrix-bound proteins. Lane 5, when included in the experiment, is a positive control NIH3T3 extract provided by Cell Signaling Technology. After sodium dodecyl sulfate–polyacrylamide gel electrophoresis, immunoassays were performed using antibodies for 4E-BP1, followed by those for eIF4E (indicated to the left). Below each lane the numbers of the *df/df* and normal mice are indicated. Also shown below each experiment is the ratio of dwarf to normal chemiluminescent signal quantified by scanning densitometry.

level. Comparisons of polysome profiles in dwarf and normal size mice also showed a lower proportion of polysomes to monosomes in dwarfs, which was restored to normal with somatotropin, but not T3, treatment (46). Messenger RNA Cap coprecipitation assays showed an increase in eIF4E-4E-BP1 binding in dwarf livers as compared to those from normal size mice. These data suggest that Cap-dependent translation is repressed in ad libitum-fed 2-month-old dwarf mouse liver, relative to tissue from normal littermates. The data on phosphorylation of S6K1 and 4E-BP1, consistent with the binding data, also indicate a down-regulation of cytoplasmic translation, as argued by Korner (45). Mammalian TOR-dependent and -independent phosphorylation of ribosomal S6 protein may not, however, be primarily involved in 5′ TOP mRNA translation, which appears to be more dependent on cues from the PI3K pathways (47). A potential reason for the variations in phosphorylation and binding activity observed in this study is variant times between death and last intake of food and the amount consumed by individual mice.

GH treatment of Snell dwarfs is associated with significant liver growth (21,28,48,49) and an increased rate of protein synthesis (48). However, no increase in liver growth was observed on IGF-1 administration, whereas other organ systems and overall length and weight showed increases (28). A separate study (50) also indicated that IGF-1 had no affect on normal liver growth. Varied IGF-1-independent effects of GH are well recognized (5–7), but one is particularly relevant to the present study regarding liver. IGF-1 null mice, which have elevated GH levels, have increased brain, kidney, heart, and liver weight, but only liver increases in size on exogenous GH treatment (51). Even more relevant to this study are the findings by van Buul-Offers and colleagues (28), showing that the size of Snell dwarf centrilobular hepatocytes increased in size in response to GH, but not IGF-1/IGF-II, treatments. Transgenic mice overexpressing bovine GH

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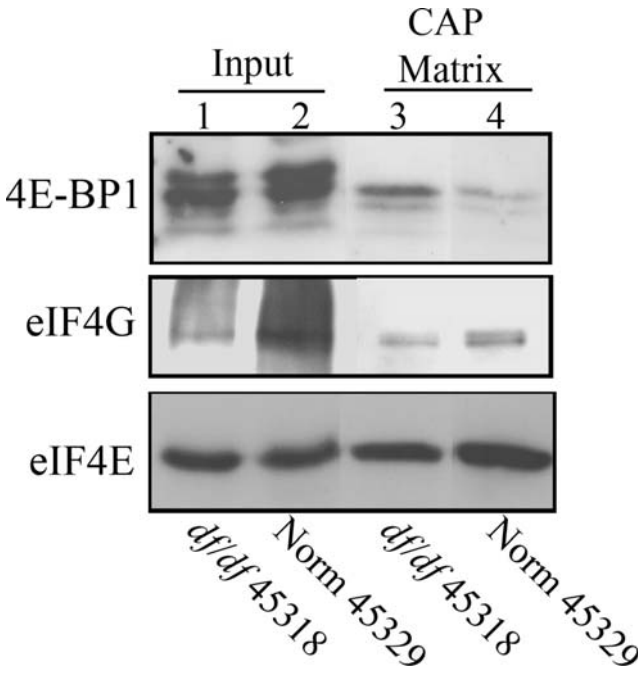

Figure 4. Decreased eIF4G coprecipitation with eIF4E in Ames dwarf liver. The binding reactions for *df/df* 45318 and normal 45329 in Figure 3 were also assayed for eIF4G. The middle panel shows the levels of eIF4G in the input (lanes 1 and 2), and that coprecipitated in the Cap-matrix-bound proteins. In this experiment, the coprecipitation of eIF4G was decreased threefold in the dwarf liver specimen.

also demonstrated hepatocellularmegaly (52,53). Because mTOR is strongly implicated in the regulation of cell size through the modulation of the translation (29,30), previous work on Snell dwarves combined with our data indicate that GH is capable of regulating translation in the mammalian liver through the PI3K/Akt/mTOR pathway.

It is interesting that the 4E-BP1 binding profile observed in dwarf livers is not seen in muscle of 2-month-old *df/df* mice. In animal and in in vitro studies, GH treatment is associated with increased protein synthesis rates and decreased breakdown of muscle (54). In humans, although an intramuscular increase in IGF-1 cannot be ruled out, local infusion of GH into muscle was associated with increased protein synthesis (54). Local administration of IGF-1 in human muscle also is associated with increased protein synthesis with little effect on breakdown (54). Two other studies suggested involvement of the PI3K/Akt/PKB/mTOR signaling systems in muscle fiber size (55) and in atrophy and hypertrophy (29). A potentially pertinent difference between liver and muscle is the rate of protein synthesis; in liver, the rate is about 16 times greater in both dwarf and normal livers compared to muscle (gastrocnemius and plantaris) (48). GH, but not IGF-1/IGF-II, stimulated growth of quadriceps femoris muscle (28,49). At this point, it can only be speculated that the reason we observe no differences is that autocrine/paracrine IGF-1 signaling in 2-month-old dwarfs is involved in the maintenance of the anabolic/catabolic balance in muscle, which at 2 months of age is similar to that in normal-size mice. It would, therefore, be interesting to compare the profiles of muscle during the earlier growth period of dwarfs and normal littermates. It would also be interesting and important to examine this differential pattern of phosphorylation in older animals.

The data on S6 kinase phosphorylation are consistent with previously reported cell biology studies, which showed that GH stimulated the phosphorylation of S6 kinase in 3T3-F442A preadipocytes (56), and is PI3K/Akt dependent (57,58). The muscle-specific pattern of S6K1 isoform phosphorylation shown here is curious in light of the different responses to GH deficiency between liver and muscle. S6K1 has been linked to the phosphorylation of other initiation factors, eIF4G and eIF4B (32,36), and we wonder, therefore, if the absence of detectable Thr389 phosphorylation of the p70 (cytoplasmic) isoform in dwarf and normal muscle may also be related to the lack of a difference in 4E-BP1 coprecipitation at 2 months of age.

Dwarf mice consume more food per gram of body weight than do normal size mice (59), thus the differences observed in the dwarfs are not likely due to a voluntary reduction in diet. A pertinent question is why dwarfs do not grow if nutrients are not lacking. In Snell dwarfs, it has been shown that T3 and/or T4 is important for amino acid uptake (48). While thyroxin increases protein synthesis in liver to a greater degree than GH, T4, unlike GH, also increases protein degradation resulting in no net growth in this organ (48). Thus, although the thyroid hormone axis is critically important in the overall, complex regulation of somatic growth, we think that the difference in 4E-BP1 coprecipitation and decreased protein synthesis in the liver are associated primarily with the reduction of GH. The findings here suggest that one possible reason for less growth in dwarf mice, at least in liver, is that PI3K/Akt/mTOR systems

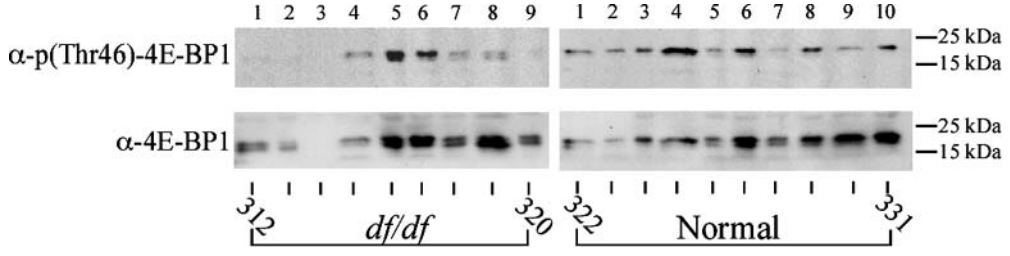

Figure 5. Phosphorylation of liver 4E-BP1 is decreased in Ames dwarf (*df/df,* mice 312-320, lanes 1–10, left) and normal-size littermates (mice 322-331, lanes 1–10, right). Liver lysates used in Figure 1 were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and were immunoassayed with the antibodies indicated on the left.

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permit less translation. Current models of the mTOR posit that it balances growth factor signaling with translation and autophagy in regulating protein mass (39). In Ames dwarfs, and probably also in Snell dwarfs, the compromised GH axis is a potentially testable explanation for the repressed state of the PI3K/Akt/mTOR-dependent translation regulatory system seen in this study.

The role of mTOR in the integration of growth factor and nutrient signaling may also be relevant to animal models of increased longevity. A prediction is that the tumor and growth suppressor, tuberous sclerosis complex (TSC), which that functions in mTOR-mediated nutrient responses (60), would be more activated in dwarf tissue relative to that of normal littermates. In addition, diet restriction of Ames dwarf mice, which results in added longevity, may further potentiate TSC growth-suppressive activities. Decreases in size associated with either diet restriction (61) or perturbation of the GH/IGF-1 axis in mice (62–64) or the insulin/IGF-1 pathways in mice (65) and fruit flies (66) results in less growth, and is beneficial to longevity. A report of extended longevity of female mice heterozygous for the IGF-I receptor knockout indicated an uncoupling of reduced size and extended longevity (67). Adipose-specific ablation of the insulin receptor resulted in decrease adipose and increased longevity (65). Restrictions of both diet and growth factor signaling in mice (68) or fruit flies (69) has additional positive affects on longevity, which may be additive at TSC or below mTOR.

Summary

These studies have provided initial evidence that low levels of GH are associated with a phosphorylation status of translation-regulatory proteins in the mTOR signaling pathways of liver and muscle of Ames dwarf mice, which are indicative of down-regulation. These findings are consistent with the previously reported lower levels of protein synthesis in these organs. Thus, the postulate developed here is that dwarf mice are small, despite eating more, because a reduction in GH-mediated signaling keeps translation and cell growth repressed. Why this phenotype is associated with longer life span is a question that might be addressed with translation inhibitors such as rapamycin. Consistent with this line of thinking are the findings that abrogation of TOR in *Caenorhabditis elegans* (70) and TOR in (or over-expression of TSC) (71) is associated with extended life spans.

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This paper is dedicated to the memory of Arun Roy, who provided advice and encouragement when it was most needed.

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