SHP-2 Regulates Cell Growth by Controlling the mTOR/S6 Kinase 1 Pathway*

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Cell growth (accumulation in cell mass) ensues through the promotion of macromolecular biosynthesis. S6 ribosomal kinase 1 (S6K1), which is activated by the mammalian target of rapamycin, is critical for cell growth. The early events that control S6K1 signaling remain unclear. Here we show that SHP-2 suppresses S6K1 activity under conditions of growth factor deprivation. We show that under conditions of growth factor deprivation, S6K1 activity was increased in fibroblasts lacking functional SHP-2 and in cells where knock down of SHP-2 expression was established by small interference RNA. Consistent with these findings, fibroblasts lacking functional SHP-2 exhibited increased cell size as compared with wild type cells. Growth factor deprivation reduces cellular energy, and the energy-sensing 5′-AMP-activated protein kinase (AMPK) negatively regulates S6K1. We found that SHP-2 promoted AMPK activity under conditions of growth factor deprivation (low energy), suggesting that SHP-2 negatively regulates S6K1 via an AMPK-dependent pathway. These results implicate SHP-2 as an early mediator in the S6K1 signaling pathway to limit cell growth in low energy states.

Environmental cues stimulate intracellular signaling pathways that regulate vital cellular functions such as cell proliferation, differentiation, migration, and apoptosis. Therefore, the ability of a cell to sense changes in environmental status is of fundamental biological importance. Mammalian target of rapamycin (mTOR)§ is an evolutionarily conserved serine/threonine kinase that has emerged as a critical sensor of environmental status. mTOR serves to integrate signals from mitogens, nutrients, and energy status to control cell growth (cell size and cell mass) and cell cycle progression (1, 2), in part through activation of S6K1 (1, 3–6).

S6K1 is a member of the AGC family of protein kinases. Recent evidence suggests that S6K1, rather than S6K2, controls cell growth (7). S6K1 is regulated by phosphorylation on multiple residues, including Thr-229 in the activation loop and Thr-389 within a conserved hydrophobic motif in the linker region (8, 9). S6K1 is directly activated by mTOR-raptor-mediated phosphorylation of Thr-389 (10–12). Phosphorylation on both residues is required for S6K1 activation, with phosphorylation at Thr-389 correlating with its kinase activity (10–13). S6K1 is thought to facilitate protein translation by positively regulating the eukaryotic elongation factor-2 (eEF2) by phosphorylating and inhibiting eEF2 kinase (14, 15). It has been proposed that the eukaryotic initiation factor 3 acts as a platform to coordinate mTOR signaling with phosphorylation of S6K1 and 4EBP-1 and subsequent S6K1 phosphorylation of the 40 S ribosomal subunit S6 and eukaryotic initiation factor 4B (16). Although the physiological requirement for S6 phosphorylation by S6K1 in protein synthesis remains unclear, its role in regulating cell size is well established (17).

There has been substantial progress in defining the molecular mechanisms that control mTOR/S6K1 signaling. Most evident is the finding that the TSC gene products TSC1 and TSC2 function as GTPase-activating proteins for the small GTPase Rheb (18). In turn, Rheb binds mTOR directly and stimulates its kinase activity (19, 20). In addition, the energy-sensing serine/threonine kinase AMPK phosphorylates and activates TSC2, resulting in the inhibition of mTOR/S6K1 under conditions of low energy (21). These findings collectively demonstrate that TSC1/TSC2, Rheb, and AMPK are upstream energy-sensing components in the mTOR/S6K1 pathway.

The reversible tyrosyl phosphorylation of intracellular signaling pathways is a key mechanism regulating cell growth and cell cycle progression. Mitogenic stimuli that activate receptor tyrosine kinases that engage the PI3K/Akt pathway also regulate the mTOR/S6K1 pathway (3). Thus, receptor tyrosine kinases are implicated in the regulation of mTOR/S6K1. Whether protein tyrosine phosphatases are involved in the regulation of mTOR/S6K1, however, remains to be determined. A potential candidate protein tyrosine phosphatase for the regulation of the mTOR/S6K1 pathway is the SH2 domain-containing protein tyrosine phosphatase SHP-2, which contains two NH2-terminal SH2 domains and a COOH-terminal protein tyrosine phosphatase domain (reviewed in Ref. 22). Multiple biochemical and genetic epistasis experiments have demonstrated that SHP-2 functions as a positive signal enhancer.
downstream of a number of receptor tyrosine kinases to regulate the Ras/Raf/ERK cascade (reviewed in Refs. 22, 23). SHP-2 also has been shown to promote activation of the PI3K signaling cascade (24–27). Hence, we speculated that SHP-2 might also be involved in the regulation of the S6K1 pathway. In this report we show that SHP-2 negatively regulates S6K1 activity and subsequently limits cell growth (accumulation of cell mass). Moreover, it appears that these effects are mediated by SHP-2 in a manner that is, at least in part, responsive to changes in energy status through AMPK.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Cell Culture**—SHP-2+/- and SHP-2Ex3-/- fibroblasts have been described previously (24). NIH 3T3 fibroblasts have been described previously (24). NIH 3T3 fibroblasts were grown as monolayers in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10% fetal bovine serum for SHP-2+/-, SHP-2Ex3-/- fibroblasts and 293 cells or 10% fetal clone III serum (HyClone, Logan, UT) for NIH 3T3 cells supplemented with 1 mM sodium pyruvate (Invitrogen), 5 units/ml penicillin, and 50 μg/ml streptomycin. Growth factor deprivation was carried out for 18–24 h in Dulbecco’s modified Eagle’s medium supplemented with 0.1% fetal bovine serum, 1 mM sodium pyruvate, 5 units/ml penicillin, and 50 μg/ml streptomycin. All chemicals were purchased from Sigma unless indicated otherwise. Rapamycin (Calbiochem, La Jolla, CA) was used at 20 nM and wortmannin at 200 nM. IGF-1 (Calbiochem) was used at 50–100 ng/ml as indicated.

**Immunoblot Analyses**—Cells were lysed in 1% Nonidet P-40 (Calbiochem) buffer containing 150 mM NaCl, 50 mM Tris-Cl, pH 7.4, 5 mM EDTA, 1% Nonidet P-40, 1 mM NaVO₃, 20 mM NaF, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, 5 μg/ml aprotinin, and 5 μg/ml leupeptin on ice. Lysates were clarified by centrifugation at 20,800 × g at 4 °C for 20 min. Protein concentration was determined using the Bradford Assay according to the manufacturer’s instructions (Pierce). For immunoblotting, cell lysates were resolved by SDS-PAGE and transferred onto Immobilon-P membranes (Millipore, Bedford, MA). All primary antibodies were used by Anthony Koleske (Yale University, New Haven, CT). Cells were cultured at 37 °C in 5% CO₂ in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10% fetal bovine serum, 1 mM sodium pyruvate, 5 units/ml penicillin, and 50 μg/ml streptomycin. Growth factor deprivation was carried out for 18–24 h in Dulbecco’s modified Eagle’s medium supplemented with 0.1% fetal bovine serum, 1 mM sodium pyruvate, 5 units/ml penicillin, and 50 μg/ml streptomycin. All chemicals were purchased from Sigma unless indicated otherwise. Rapamycin (Calbiochem, La Jolla, CA) was used at 20 nM and wortmannin at 200 nM. IGF-1 (Calbiochem) was used at 50–100 ng/ml as indicated.

**Enzyme-Linked Immunosorbent Assay**—Cells were collected, pelleted by centrifugation at 10,000 × g, and resuspended in Dulbecco’s modified Eagle’s medium supplemented with either 0.1% or 10% fetal bovine serum (Sigma), 1 mM sodium pyruvate (Invitrogen), 5 units/ml penicillin, and 50 μg/ml streptomycin (Sigma). Cell number was determined using a BD Biosciences Coulter Multisizer. Statistical significances were calculated by performing a two-tailed t test assuming unequal variances.

**Adenoviral Infections and siRNA Transfections**—Recombinant wild type (WT) and catalytically inactive (ΔP and RM) adenoviruses have been described previously (28). Cells were infected at 5 × 10⁶ optical particle unit/1 × 10⁶ cells. Synthetic siRNA duplexes were purchased from Dharmacon, Inc. (Lafayette, CO) and targeted the SHP-2 sequence 5’-CCCCAAAAGAGUUACAUUGGC-3’. The control siRNA is siCONTROL, a non-targeting siRNA (D-001210–01; Dharmacon). NIH 3T3 cells were transfected with either 50 nM SHP-2 siRNA or siCONTROL using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instruction. After 2 days, cells were growth factor-deprived for an additional 18–24 h before immunoblot analysis.

**Transient Transfections and Cell Sorting**—Cells were transfected with pIRES, pIRES-SHP-2(myc), or pIRES-SHP-2-EA(myc) plasmid DNA using Lipofectamine 2000. The next day, cells were rinsed three times with phosphate-buffered saline before growth factor deprivation. After an additional 24 h, cells were collected by trypsinization and GFP-positive cells were sorted using a BD Biosciences flow cytometer.

**S6K1 in Vitro Kinase Assays**—S6K1 was immunoprecipitated using the C3 antibody and protein A-Sepharose. Immune complexes were washed twice with 1% Nonidet P-40 lysis buffer, once with STE (150 mM NaCl, 50 mM Tris-Cl, pH 7.4, 5 mM EGTA), and twice with kinase wash buffer (20 mM Hepes, pH 7.4, 10 mM MgCl₂). Kinase reactions were carried out using glutathione S-transferase-S6 as a substrate for 12 min at 30 °C in kinase reaction buffer (20 mM Hepes, pH 7.4, 10 mM MgCl₂, 3 ng/μl protein kinase A inhibitor, 50 μM ATP, 10 μCi [γ-32P]ATP). Reactions were stopped by the addition of sample buffer and heating at 95 °C for 5 min.

**RESULTS**

**SHP-2 Negatively Regulates S6K1 Activity**—SHP-2 is required for the activation of the PI3K/Akt pathway (24–27). Therefore, we hypothesized that SHP-2 might play a role in PI3K-mediated signaling to positively regulate S6K1 activity and...
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A

**FIGURE 1. Negative regulation of S6K1 by SHP-2.** A, SHP-2
transfected fibroblasts were growth factor-deprived and then either left unstimulated or restimulated for 10 min with IGF-1 (50 ng/ml). Cells were lysed and subjected to SDS-PAGE followed by immunoblotting with SHP-2, pS6K1 and S6K1, pERK and ERK, or pAkt and Akt antibodies. B, SHP-2
transfected fibroblasts were treated as in panel A. Where indicated, cells were pretreated with rapamycin (Rap) for 30 min before stimulation with IGF-1 (50 ng/ml). S6K1 was immunoprecipitated, and immune complexes were subjected to an in vitro kinase assay using glutathione S-transferase-S6 as a substrate. Graph below shows normalized S6K1 activity (GST-S6/S6K1). C, SHP-2
transfected and SHP-2
transfected fibroblasts were treated as in panel A for the indicated times with IGF-1 (50 ng/ml). Cells were lysed and subjected to SDS-PAGE, followed by immunoblotting with pS6K1 and S6K1 antibodies. Normalized pS6K1 levels shown below represent the densitometric units of the ratio between pS6K1 and S6K1. D, fibroblasts transfected with either 50 nm siRNA to SHP-2 or a non-targeting (NT) siRNA control; cell lysates were immunoblotted with the indicated antibodies.

subsequently promote cell growth (cell size and cell mass). We first determined whether S6K1 activation is perturbed in SHP-2
transfected fibroblasts following stimulation with IGF-1. SHP-2
transfected fibroblasts contain a deletion within exon 3 of SHP-2 resulting in the truncation of the amino-terminal SH2 domain (29). In all cases examined, SHP-2
transfected fibroblasts behave phenotypically as SHP-2 loss-of-function cells (22). S6K1 activation was assessed as a function of Thr-389 phosphorylation. As shown previously (24, 26), SHP-2
transfected fibroblasts were inhibited in their ability to stimulate Akt and ERK activation in response to IGF-1 as compared with SHP-2
transfected fibroblasts (Fig. 1A). SHP-2
transfected fibroblasts induced S6K1 phosphorylation in response to IGF-1, as expected (Fig. 1A). In contrast, SHP-2
transfected fibroblasts appeared to be less responsive to IGF-1-induced S6K1 phosphorylation; this was presumably due to the observation that these cells had markedly higher levels of S6K1 phosphorylation under growth factor-deprived conditions as compared with SHP-2
transfected fibroblasts (Fig. 1A). This result was supported by immune complex S6K1 activity assays that also demonstrated that SHP-2
transfected fibroblasts exhibited increased S6K1 activity when growth factor-deprived (Fig. 1B). As expected, IGF-1-inducible S6K1 activity was sensitive to rapamycin in both SHP-2
transfected and SHP-2
transfected fibroblasts (Fig. 1B). Next, we examined S6K1 phosphorylation during G1 progression to establish the kinetics of S6K1 activation in SHP-2
transfected fibroblasts. As shown in Fig. 1C, SHP-2
transfected fibroblasts displayed a transient increase in S6K1 phosphorylation following IGF-1 stimulation. In SHP-2
transfected fibroblasts, S6K1 phosphorylation was elevated prior to IGF-1 stimulation (Fig. 1C). When SHP-2
transfected fibroblasts were released into G1 upon IGF-1 stimulation, S6K1 phosphorylation increased further and remained elevated throughout G1 (Fig. 1C).

To further substantiate the argument that SHP-2 suppresses S6K1 phosphorylation under conditions of growth factor deprivation, the expression of SHP-2 was depleted using siRNA. Transfection of 3T3 fibroblasts with siRNA oligonucleotides reduced SHP-2 expression (Fig. 1D). Accordingly, SHP-2 siRNA-treated fibroblasts were impaired in their ability to activate ERK in response to IGF-1 stimulation (Fig. 1D). S6K1 phosphorylation in SHP-2 siRNA-treated fibroblasts under conditions of growth factor deprivation enhanced S6K1 phosphorylation (Fig. 1D). Collectively, these results demonstrate that SHP-2 negatively regulates S6K1 phosphorylation under conditions of growth factor deprivation and during G1 progression.
but not rapamycin (Fig. 2). These results suggest that in growth factor-deprived cells SHP-2 lies upstream of, and/or parallel to, both PI3K and mTOR in the control of S6K1 phosphorylation.

We next attempted to rescue the enhanced S6K1 phosphorylation in growth factor-deprived SHP-2<sup>Ex3−/−</sup> fibroblasts. In Fig. 3A, we show that this defect could be rescued by reintroduction of wild type SHP-2 into SHP-2<sup>Ex3−/−</sup> fibroblasts; S6K1 phosphorylation in wild type SHP-2 expressing SHP-2<sup>Ex3−/−</sup> fibroblasts was reduced to levels that were undetectable as compared with GFP adenoviral infected control cells. In addition, expression of a constitutively active mutant of SHP-2 (SHP-2.E76A) into SHP-2<sup>Ex3−/−</sup> fibroblasts also suppressed S6K1 activity relative to GFP control infectants under conditions of growth factor deprivation (Fig. 3A). These results support the interpretation that SHP-2 negatively regulates S6K1 in growth factor-deprived cells.

To address whether SHP-2 inhibition of S6K1 phosphorylation in response to growth factor deprivation was a function of SHP-2 phosphatase activity, we infected 3T3 fibroblasts with recombinant adenoviruses expressing two distinct catalytically inactive mutants of SHP-2 that serve as dominant-negatives; SHP-2(ΔP) contains a deletion within the phosphatase domain (30), and SHP-2(RM) is an Arg-495 to Met-495 mutation within the protein tyrosine phosphatase domain (31). Under conditions of growth factor deprivation, GFP control infected 3T3 fibroblasts exhibited low levels of S6K1 activity (Fig. 3B), whereas cells infected with either SHP-2(ΔP) or SHP-2(RM) had increased levels of S6K1 activity in growth factor-deprived fibroblasts to levels similar to that of IGF-1-inducible S6K1 phosphorylation (Fig. 3B). These data indicate that the catalytic activity of SHP-2 is necessary for the suppression of S6K1 under conditions of growth factor deprivation.

**SHP-2 Negatively Regulates Cell Size**—S6K1 is a critical regulator of cell size (3, 5, 9). Because SHP-2 negatively regulates S6K1 phosphorylation we determined whether this resulted in an increase in the size of SHP-2<sup>Ex3−/−</sup> fibroblasts. SHP-2<sup>+/+</sup> and SHP-2<sup>Ex3−/−</sup> fibroblasts were growth factor-deprived for 72 h, and cell size was determined. SHP-2<sup>Ex3−/−</sup> fibroblasts were found to be larger in size than SHP-2<sup>+/+</sup> fibroblasts as depicted by a rightward shift in the size distribution of these cells (Fig. 4A, left panel). Quantitation of these data showed that there was a significant increase, ~10%, in the size of SHP-2<sup>Ex3−/−</sup> fibroblasts as compared with SHP-2<sup>+/+</sup> fibroblasts (Fig. 4A, right panel). Similar results were obtained under proliferating conditions (data not shown).

We next sought to demonstrate that the increased cell size of SHP-2<sup>Ex3−/−</sup> fibroblasts was due specifically to the loss of functional SHP-2. SHP-2<sup>Ex3−/−</sup> fibroblasts were transected with GFP control, GFP-SHP-2 (WT), or GFP-SHP-2 (EA). Growth factor-deprived GFP-positive SHP-2<sup>Ex3−/−</sup> fibroblasts were collected by cell sorting using flow cytometry, and the diameter of those cells was analyzed. SHP-2 (WT) and SHP-2 (EA) were expressed to equivalent levels (Fig. 4B). Expression of SHP-2 (WT) in SHP-2<sup>Ex3−/−</sup> fibroblasts resulted in a reduction in cell size as compared with GFP control-expressing cells (Fig. 4B). Additionally, SHP-2<sup>Ex3−/−</sup> fibroblasts expressing the constitutively active mutant of SHP-2 (EA) exhibited a more pronounced decrease in cell size as compared with SHP-2<sup>Ex3−/−</sup> fibroblasts expressing SHP-2 (WT) (Fig. 4B). Next, we used rapamycin to test whether the increased cell size of SHP-2<sup>Ex3−/−</sup> fibroblasts was dependent upon the activity of mTOR. These results showed that rapamycin significantly attenuated the increased cell size in SHP-2<sup>Ex3−/−</sup> fibroblasts (Fig. 4C), arguing that SHP-2 negatively regulates cell size either upstream of, and/or parallel to, mTOR.

Consistent with the larger size of SHP-2<sup>Ex3−/−</sup> fibroblasts (Fig. 4), we found that these cells had significantly increased levels of protein content per cell as compared with SHP-2<sup>+/+</sup> fibroblasts (Fig. 5A). In mammalian cells, protein synthesis is...
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**SHP-2 Is Required for AMPK-dependent Suppression of S6K1**—Energy sensing is mediated through the actions of the 5'-AMPK that is activated in response to reduced energy availability (34, 35). Growth factor deprivation results in the depletion of cellular ATP and hence an increase in the AMP/ATP ratio (36). AMPK when activated by an increase in the AMP/ATP ratio phosphorylates TSC2, enhancing its GTPase activity, thereby inhibiting mTOR/S6K1 (21, 37, 38). We therefore hypothesized that under conditions of growth factor deprivation SHP-2 promotes AMPK activity and subsequently inactivation of S6K1. AMPKα activity levels were assessed by measuring the phosphorylation status of its regulatory T-loop phosphorylation site (Thr-172) (39) in SHP-2-Ex3−/− and SHP-2+/+ fibroblasts. Under conditions of growth factor deprivation (lowered energy status), AMPK phosphorylation in SHP-2-Ex3−/− fibroblasts was found to be markedly lower as compared with SHP-2+/+ fibroblasts (Fig. 6A). AMPK phosphorylation was stimulated in SHP-2-Ex3−/− fibroblasts upon treatment with 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) which activates AMPK in an LKB1-dependent manner (Fig. 6A). Significantly, AICAR treatment suppressed the enhanced level of S6K1 phosphorylation in SHP-2-Ex3−/− fibroblasts (Fig. 6B). These results support the interpretation that SHP-2 promotes AMPK activity, providing a potential link between SHP-2 and its ability to suppress S6K1.

**DISCUSSION**

S6K1 is a major effector of mTOR, and mice lacking S6K1 are reduced in size at birth (40, 41), demonstrating a role of S6K1 in the regulation of cell and organism size. We have found that SHP-2 suppresses S6K1 activity under conditions of growth factor deprivation in SHP-2 loss-of-function fibroblasts and by knock down of SHP-2 expression in fibroblasts by siRNA. Further, the enhanced level of S6K1 activity was rescued by re-introduction of wild type SHP-2 into SHP-2-Ex3−/− fibroblasts. Importantly, we show that under conditions of growth factor deprivation overexpression of a catalytically inactive mutant of SHP-2 was sufficient to enhance S6K1 activity. During G1 pro-
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S6K1 activity was also enhanced in SHP-2<sup>Ex3</sup>−/− fibroblasts, suggesting that SHP-2 negatively regulates S6K1 during the G<sub>1</sub> phase of the cell cycle. Consistent with these observations we found that SHP-2<sup>Ex3</sup>−/− fibroblasts were larger in size than wild type fibroblasts. Together, these results demonstrate that SHP-2 functions to attenuate cell size by limiting S6K1 activity in growth factor-deprived cells and during cell cycle progression.

The observation that SHP-2 suppresses S6K1 activity was somewhat surprising because we and others have shown that SHP-2 is required for activation of the PI3K pathway (24–27) that promotes S6K1 activation. However, the enhanced levels of S6K1 activity were inhibited by wortmannin. It remains possible that a wortmannin-sensitive PI3K family member might contribute to the regulation of S6K1 downstream of SHP-2. In this regard, it has been shown that mTOR signaling by amino acids can be mediated by the class 3 PI3K family member hVps34 (42, 43). Whether SHP-2 regulates hVps34 signaling in the control of nutrient and/or energy-sensing activation of S6K1 remains to be determined.

SHP-2<sup>Ex3</sup>−/− fibroblasts were found to be significantly larger in size as compared with wild type fibroblasts. Rapamycin rescued this defect, providing support for the assignment of SHP-2 acting either upstream or, and/or parallel to, mTOR. SHP-2<sup>Ex3</sup>−/− fibroblasts contained increased levels of protein/cell as compared with wild type fibroblasts, consistent with the enhanced S6K1 activity and increased activity of eEF2 (14, 32, 33). These data are consistent with the interpretation that under growth factor-deprived conditions SHP-2 limits protein translation by inhibiting S6K1. Because the elongation step in protein translation consumes a large amount of energy, suppression of S6K1 activity by SHP-2 under conditions of growth factor deprivation (where energy levels are low) would retard elongation and thus save energy.

Both S6K1 and SHP-2 are required for cell cycle progression (4, 44). When cells proliferate, cell size must be strictly maintained, and once a cell has “registered” its correct size, further growth is attenuated (2). Failure to regulate cell growth coordinately with cell cycle progression is critical for faithful cell division. Interestingly, SHP-2 is uncoupled from its positive signaling effects to ERK late in G<sub>1</sub> (44). Therefore, SHP-2 might serve to limit cell growth by suppressing S6K1 activity at the later stages of G<sub>1</sub> as part of a temporally distinct signaling pathway (independent of ERK) during cell proliferation. We also found that a constitutively active mutant of SHP-2 had a more potent effect in rescuing the increased cell size in SHP-2<sup>Ex3</sup>−/− fibroblasts than wild type SHP-2. This suggests that the catalytic activity of SHP-2 is sufficient to suppress cell size. Consistent with this we have observed that fibroblasts derived from mice harboring the Noonan syndrome-associated mutation D61G in which SHP-2 is constitutively active (45) also appear to be smaller than wild type fibroblasts (data not shown). Provocatively, the D61G Noonan syndrome knock-in mice (45), as well as patients harboring Noonan syndrome-associated SHP-2-activating mutations, exhibit proportionate short stature. Whether the mTOR/S6K1-signalizing axis is disrupted in Noonan syndrome-associated SHP-2 mutant mice requires further investigation.

Our data reveal a potential link through which SHP-2 negatively regulates S6K1. It has been shown that AMPK activity inhibits S6K1 via direct phosphorylation of TSC2 (21). AMPK, which is regulated by changes in the AMP:ATP ratio (34, 35), is reduced in its level of phosphorylation in SHP-2<sup>Ex3</sup>−/− fibroblasts as compared with wild type cells when growth factor levels are low. Given that growth factor deprivation results in the depletion of cellular energy (36), our results implicate a role for SHP-2 in energy sensing. How SHP-2 serves to sense energy changes is unclear; SHP-2 has been found to be expressed in mitochondria, suggesting a potential role for SHP-2 in the management of energy status (46). Treatment of SHP-2<sup>Ex3</sup>−/− fibroblasts with 5-aminimidazole-4-carboxamide riboside rescues the enhanced S6K1 activity, demonstrating that SHP-2 is unlikely to be controlling AMPK in a LKB1-dependent manner, consistent with the current perception that LKB1 does not appear to be regulated.

S6K1 activates eEF2 kinase by direct phosphorylation. eEF2 kinase then phosphorylates and inactivates eEF2 (14, 32, 33). In the absence of SHP-2, eEF2 phosphorylation is reduced, which supports the interpretation that SHP-2 attenuates protein translation. These data are also in line with the observation that AMPK phosphorylates and activates eEF2 kinase (47). In light of our recent findings that demonstrate that SHP-2 regulates Ca<sup>2+</sup> signaling (48), a potential SHP-2 target for the regulation...
of AMPK could be the Ca\(^{2+}\)-calmodulin-dependent kinase kinase β (49, 50). However, we cannot rule out the possibility that SHP-2 might also regulate mTOR/S6K1 signaling via an AMPK-independent mechanism. Further studies will be required to fully define the mechanism of SHP-2 signaling in the energy-sensing pathway. In particular, it will be important to understand how SHP-2 activity is regulated by changes in energy status and subsequently the substrates dephosphorylated by SHP-2 in this pathway.

In summary, we define a novel function for SHP-2 in the regulation of S6K1 signaling whereby SHP-2 acts to coordinate the early signaling events involved in the regulation of macromolecular biosynthesis.

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REFERENCES


FIGURE 6. Regulation of AMPK activity by SHP-2. A, SHP-2\(^{+/+}\) and SHP-2\(^{-/-}\) fibroblasts were growth factor-deprived and were either left untreated or exposed to 5-aminomidazole-4-carboxamide riboside (AICAR) (1 mM) for 1 h. Cells were immunoblotted with pAMPK and AMPK antibodies. A representative experiment with corresponding densitometric analysis of pAMPK/AMPK levels is shown. B, lysates from panel A immunoblotted with pS6K1 and S6K1 antibodies. A representative experiment with corresponding densitometric analysis of pS6K1/S6K levels is shown.
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