A-kinase anchoring protein 8L interacts with mTORC1 and promotes cell growth

Chase H. Melick1,2,3, Delong Meng1,2,3, Jenna L. Jewell1,2,3

Affiliations:
1 Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.
2 Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.
3 Hamon Center for Regenerative Science and Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA.

Running title: AKAP8L mediates protein translation

*To whom correspondence should be addressed: Dr. Jenna L. Jewell, Ph.D. Department of Molecular Biology, University of Texas Southwestern Medical Center, 6000 Harry Hines Blvd. Room# NA5.508A, Dallas, Texas, 75390-9148. Tel: (214) 648-1685. Fax: (214) 648-1488 Email: Jenna.Jewell@UTSouthwestern.edu

Keywords: mTORC1, A-kinase anchoring protein (AKAP), mRNA translation, cell size, cell proliferation, AKAP8L, nutrient sensing, scaffolding protein, anabolic pathway, cAMP signaling

Abstract

The mammalian target of rapamycin complex 1 (mTORC1) senses nutrients to mediate anabolic processes within the cell. Exactly how mTORC1 promotes cell growth remains unclear. Here, we identified a novel mTORC1-interacting protein called A-kinase anchoring protein 8L (AKAP8L). Using biochemical assays, we found that the N-terminal region of AKAP8L binds to mTORC1 in the cytoplasm. Importantly, loss of AKAP8L decreased mTORC1-mediated processes such as translation, cell growth and cell proliferation. AKAPs anchor protein kinase A (PKA) through PKA regulatory subunits, and we show that AKAP8L can anchor PKA through regulatory subunit Iα. Reintroducing full-length AKAP8L into cells restored mTORC1-regulated activities, whereas reintroduction of AKAP8L missing the N-terminal region that confers the interaction with mTORC1 did not. Our results suggest a multifaceted role for AKAPs in the cell. We conclude that mTORC1 appears to regulate protein translation, perhaps in part through AKAP8L.

Nutrients promote anabolic pathways like protein synthesis in order to increase cell size and proliferation. Alternatively, when nutrients are limiting, catabolic processes such as autophagy are initiated to produce energy for the cell. mTORC1 controls these events by sensing nutrient availability and is often referred to as a “master regulator” of cell growth (1-4). mTORC1 is comprised of three main components: the catalytic subunit, mTOR, Raptor (the substrate recognizing component) and mLST8 (a positive regulator of mTORC1). In the current model, increased nutrients such as amino acids promote mTORC1 lysosomal localization and subsequent activation (5,6). When mTORC1 is at the lysosome it binds to and is activated by the small G-protein, Ras homolog enriched in brain (Rheb), downstream of growth factor signaling. Once activated, mTORC1 can control multiple downstream processes such as protein translation (3,4,7-9).

mTORC1 is known to control protein translation through the phosphorylation of two well-characterized substrates, ribosomal S6 kinases (S6Ks) and the eIF4E-binding proteins (4EBPs) (1,10,11). S6K modulates translation initiation factors and ribosome biogenesis through elf4B and S6 (12). In studies using mTORC1 active-site inhibitors (PP242 and Torin1), 4EBPs were found to play a major role in regulating cell proliferation (13). Specifically, 4EBPs inhibit
AKAP8L mediates protein translation

Through the eukaryotic translation initiation factor 4F (eIF4F) complex assembly by associating with eIF4E (14,15). 4EBPs have also been implicated in the regulation of specific mTORC1 regulated transcripts, known as pyrimidine-rich 5' TOP or “TOP-like” motifs (16-18). These TOP mRNAs are responsible for encoding ribosomal proteins and elongation factors. La-related protein 1 (LARP1), also modulates TOP mRNAs, and a recent study revealed that LARP1 is a direct substrate of mTORC1 (19-22). The phosphorylation of LARP1 by mTORC1 results in LARP1 dissociation from the 5' untranslated region (UTR) of ribosomal protein (RP) mRNAs. LARP1 acts as a molecular switch for the activation or repression of specific mTORC1 substrates that regulate protein translation. Additional mTORC1 substrates, like UNC-5 like autophagy activating kinase 1 (ULK1), are involved in other biological processes like autophagy (23).

G-protein coupled receptors (GPCRs) are members of one of the most widely therapeutically targeted protein families, consisting of about 34% of all FDA approved drugs (24). GPCRs mediate specific downstream signaling cascades depending on the specific G-protein coupled to the receptor (24-27). GPCRs coupled to Gαs proteins can regulate downstream targets through the secondary messenger 3', 5'-cyclic adenosine monophosphate (cAMP) (28). Elevated levels of cAMP activate PKA which then inhibits mTORC1 (45). PKA directly phosphorylates the mTORC1 component Raptor on Ser 791, leading to inhibition of mTORC1 activity in multiple cell lines and mouse tissue. Thus, we are actively searching for other machinery involved in the cross-talk between mTORC1 and PKA.

Here, we identify AKAP8L as a new mTORC1 binding protein in the cytoplasm. Although AKAP8L does not alter the phosphorylation of Raptor at Ser 791 leading to mTORC1 inhibition, AKAP8L appears to play a crucial role in mTORC1-mediated processes. AKAP8L promotes protein translation, cell size and proliferation. Moreover, we found that the N-terminal region of AKAP8L, which is required for the interaction with mTORC1, is critical in mediating these events.

Results
AKAP8L interacts with mTORC1

To identify potential mTORC1 interacting proteins, we expressed Flag-tagged Raptor in HEK293A cells and analyzed anti-Flag immunoprecipitates by mass spectrometry. We identified AKAP8L as one of the hits. To confirm that AKAP8L interacts with mTORC1, we performed co-immunoprecipitation experiments. Interestingly, AKAP8L mediated the interaction between Flag-tagged AKAP8L and HA-tagged Raptor under normal cell culturing conditions. Additionally, a reverse immunoprecipitation experiment revealed that Flag-tagged AKAP8L could co-immunoprecipitate HA-tagged Raptor.
AKAP8L mediates protein translation

AKAP8L and HA-tagged Raptor increases compared to normal conditions (Supplemental Fig. 1). Moreover, endogenous AKAP8L was able to interact with both endogenous Raptor (Fig. 1C), and an overexpressed HA-tagged Raptor (Fig. 1D). AKAP8L and homologous relative AKAP8 contain 61% sequence identity (Fig. 1E) (46). Both AKAP8L and AKAP8 have zinc finger motifs and mono and bipartite nuclear localization sequences (NLS). AKAP8L also contains a nuclear export sequence (NES), a YG domain of unknown function, an FG domain that resembles nuclear pore-like repeats (47) and a proline rich region similar to a SRC Homology 3 (SH3)-binding domain (48,49). The proline rich domain on AKAP8L exists in a similar area as the AKAP8 RII binding region. Although AKAP8L interacts with Raptor, AKAP8 does not (Fig. 1C). Flag-tagged AKAP8L interacted with the mTORC1 components mTOR, mLST8 and PRAS40 (Fig. 1F). Thus, AKAP8L is an mTORC1 interacting protein.

AKAP8L interacts with mTORC1 through the N-terminus

In order to determine the region on AKAP8L that could interact with Raptor, we generated several AKAP8L truncations. We designed and generated 8 different AKAP8L truncations similar to that of another study (Fig. 2A) (49). HA-tagged Raptor was co-expressed with either full length Flag-tagged AKAP8L (1-646), Flag-tagged N-terminal truncations of AKAP8L (63-646 or 247-646) or C-terminal truncations of AKAP8L (1-551, 1-384, 1-349, 1-279, 1-268, or 1-247) (Fig. 2B). Flag-tagged AKAP8L and the truncations of Flag-tagged AKAP8L were expressed at similar levels in the whole cell lysate (WCL) (Fig. 2B – bottom panel). Immunoprecipitation experiments of HA-tagged Raptor showed that full length Flag-tagged AKAP8L and most of the Flag-tagged AKAP8L truncations could still interact with HA-tagged Raptor (Fig. 2B – top panel). However, Flag-tagged AKAP8L 247-646 was unable to bind to Raptor, indicating that the N-terminus of AKAP8L is critical for the interaction with mTORC1. Because Flag-tagged AKAP8L 63-646 could still bind to mTORC1, we narrowed down amino acids 63-247 of AKAP8L to be important for the AKAP8L-mTORC1 interaction. To test if amino acids 63-247 on AKAP8L alone are capable of binding to Raptor, we overexpressed Flag-tagged AKAP8L 63-246 with HA-tagged Raptor (Fig. 2C). Amino acids 63-247 of AKAP8L was sufficient to bind to Raptor. Taken together, these results suggest that amino acids 63-247 of AKAP8L are necessary and sufficient for interaction with mTORC1.

AKAP8L – mTORC1 interaction occurs in the cytoplasm

AKAP8L has been shown to localize to the nucleus (46,50,51). However, another study has reported that AKAP8L can also reside in the cytoplasm (49,52). AKAP8 localizes to the nucleus and has no known NES or role in the cytoplasm (53,54). We confirmed that AKAP8L localizes both to the nucleus and cytoplasm by overexpressing Flag-tagged AKAP8L (red) in mouse embryonic fibroblasts (MEFs), followed by immunofluorescence (Fig. 3A). AKAP8L appears to not co-localize with the tested organelle markers calreticulin (endoplasmic reticulum), Cox IV (mitochondria), LAMP2 (lysosome), and GM130 (Golgi apparatus) (Supplementary Fig. 2). Complimentary experiments using subcellular fractionation confirmed that Flag-tagged AKAP8L and endogenous AKAP8L localized to both the nucleus and cytoplasm (Fig. 3B-C). To determine where the Raptor-AKAP8L interaction occurred, we performed nuclear and cytoplasmic isolation experiments followed by immunoprecipitation of HA-tagged Raptor (55) (Fig. 3D). HA-tagged Raptor bound to endogenous AKAP8L in the cytoplasm. Thus, AKAP8L interacts with mTORC1 in the cytoplasm and not the nucleus.

We recently showed that PKA inhibits mTORC1 activity directly through the phosphorylation of Raptor at Ser 791 (45). Because AKAPs typically anchor PKA to distinct regions of the cell through regulatory subunits, we tested whether Raptor could form a complex with AKAP8L and the catalytic and regulatory subunits of PKA. Previously, AKAP8L was shown not to associate with PKA regulatory subunit II α in vitro (46). Consistently, Flag-tagged AKAP8L did not interact with mCherry-tagged PKA regulatory subunit II α in cells (Fig. 3E). Interestingly, Flag-tagged AKAP8L could bind to PKA regulatory subunit II α and the PKA catalytic subunit (Fig. 3F). Thus, mTORC1 can form a complex with AKAP8L, PKA regulatory subunit II α, and PKA catalytic subunit. To determine whether AKAP8L...
AKAP8L mediates protein translation

is involved in the phosphorylation of Raptor at Ser 791, we overexpressed HA-tagged Raptor with or without Flag-tagged AKAP8L (Supplemental Fig. 3A). Cells were treated with or without forskolin, HA-tagged Raptor was immunoprecipitated, and assessed for Raptor Ser 791 phosphorylation via a phospho-PKA substrate antibody. A commercially available antibody recognizes phosphorylated proteins on Ser or Thr residues within the PKA recognition motif R-R-X-S*/T*. We previously showed that it is specific for Raptor Ser 791 phosphorylation (45). There was no change in Raptor Ser 791 phosphorylation when Flag-tagged AKAP8L was over expressed. Overexpression of full-length Flag-AKAP8L or the region of AKAP8L that binds to mTORC1 (Flag-AKAP8L 63-247) did not alter the phosphorylation of known mTORC1 substrates (Supplemental Fig. 3B-C, Supplemental Fig. 4A). Likewise, depletion of AKAP8L did not change the mTORC1 activity via the phosphorylation of known mTORC1 substrates (Supplemental Fig. 3D-E, Supplemental Fig. 4B-C). Taken together, AKAP8L does not appear to regulate the phosphorylation of Raptor Ser 791 or the phosphorylation of the mTORC1 substrates S6K, 4EBP1, LARP1, or ULK1.

mTORC1 regulates many cellular processes, including protein translation. Additionally, studies in yeast have found that cAMP levels modulate cell size (56), and rate of protein synthesis for cell division (57), and inhibits translation in mammalian cells (58). Recent work from our lab demonstrated that increasing cAMP levels could inhibit global protein translation in mammalian cells (45). Therefore, we were interested in investigating if AKAP8L and the mTORC1-AKAP8L interaction had a role in translation. AKAP8L has been implicated in transcription (59) and was observed to interact with RNA helicase A (RHA), also known as DHX9 (49,52). Interestingly, RHA has been suggested to partake in the translation process of highly structured RNAs (60,61) and was shown to recruit to the 5’ mRNA cap structure under mTORC1 activating conditions (20). Therefore, we investigated whether RHA could be involved in our mTORC1-AKAP8L interaction. RHA has been thought to bind AKAP8L at the N-terminus and C-terminus (49). Indeed, we confirmed that HA-tagged RHA could interact with Flag-tagged AKAP8L (Supplementary Fig. 5A). Next, we overexpressed increasing amounts of HA-tagged RHA to determine if RHA could disrupt the Raptor-AKAP8L interaction (Supplemental Fig. 5B). HA-tagged RHA did not complex with immunoprecipitated Myc-tagged Raptor nor disrupt Flag-tagged AKAP8L binding. Thus, RHA does not appear to partake in this interaction, in this context.

AKAP8L promotes mTORC1 mediated biology

Next, we investigated whether AKAP8L could play a role in translation and mTORC1-mediated biology. To test if AKAP8L is essential in mTORC1 mediated biology, we generated 2 different AKAP8L knock out (KO) HEK293A cells using 2 different guide RNAs (sgRNAs) via CRISPR-Cas9 system (Supplemental Fig. 6A-B). HEK293A cells contain at least 3 copies of AKAP8L. All indels in the AKAP8L KO clonal cell lines resulted in frame-shift mutations. Loss of AKAP8L protein expression was further confirmed by immunoblotting with an AKAP8L antibody. Interestingly, AKAP8L KO cells have a significant reduction in global protein translation when compared to control cells (Fig. 4A). A widely used technique in the mTORC1 field to measure actively translating mRNAs is polysome profiling (13,17,18,62), which is based on the separation of translated mRNAs associated with polysomes compared to untranslated mRNAs. Similar to global protein translation, a loss of AKAP8L lead to a reduction in actively translating mRNAs shown by a decrease in the polysome fraction (Fig. 4B). It has previously been reported that mTORC1 inhibition results in the reduction of nearly all mRNAs to some extent; as expected, inhibition of mTORC1 with the ATP mimetic Torin1 also decreased the polysome fraction (17). To confirm that the loss of protein translation is indeed due to the deletion of AKAP8L, we stably overexpressed Flag-tagged AKAP8L in AKAP8L KO HEK293A cells and found that it could rescue the polysome fraction (Fig. 4B, Supplemental Fig. 6C, Supplemental Fig. 7). mTORC1 is a critical regulator of cell size and proliferation (63,64). Because a loss of AKAP8L lead to translation reduction, we examined the role of AKAP8L in cell size and proliferation. Indeed, a loss of AKAP8L significantly reduced cell size (Fig. 4C and Supplementary Fig. 8A) and cell proliferation (Fig. 4D and Supplementary Fig.
AKAP8L mediates protein translation 8B). Importantly, overexpression of Flag-tagged full length AKAP8L, but not a mTORC1 defective binding mutant of AKAP8L (Flag AKAP8L 247-646), could rescue cell size and proliferation (Fig. 4C-D and Supplementary Fig. 6D). Thus, AKAP8L and the region of AKAP8L that binds to mTORC1 are important for promoting mTORC1-mediated biology.

Discussion

It is well-established that mTORC1 controls cell growth (65). Though the mechanism of this regulation has not been fully elucidated, key substrates of protein translation like 4EBP1 (14,15,66), S6K (18,63,64,67), and LARP1 (19-22) have been discovered to regulate this process (1,9). Here, we report a previously unidentified interaction between mTORC1 and AKAP8L. We demonstrate that amino acids 63-247 of AKAP8L bind to mTORC1. Moreover, the subcellular localization of this AKAP8L-mTORC1 interaction resides in the cytoplasm. We also find that AKAP8L can complex with the PKA holoenzyme, through regulatory subunit 1α. Deletion of AKAP8L in cells led to a reduction in protein synthesis, cell size and proliferation. Furthermore, rescuing AKAP8L knockout cells with the full-length protein, restored mTORC1 mediated processes. AKAP8L protein missing the N-terminal region, crucial to bind mTORC1, did not rescue mTORC1 biology. We propose that the mTORC1-AKAP8L interaction has an important role promoting anabolic processes like translation, through an unknown molecular mechanism (Fig. 4E).

An RNA binding protein called S6K1 Aly/REF-like substrate (SKAR) (68,69), has been reported to serve as a scaffolding protein between S6K1 and newly spliced mRNA (69). SKAR has been shown to regulate cell growth, as siRNA knockdown of SKAR caused a reduction in cell size (68). Similar to SKAR, we found that AKAP8L knockout cells experienced significant decrease in cell size. Like SKAR, it could be possible that AKAP8L serves as a scaffold for components involved in translation and cell growth. This could explain why the phosphorylation of known mTORC1 substrates were unaffected, yet growth was impacted. Alternatively, AKAP8L may be involved in active translation through RHA (Supplemental Fig. 5A).

mRNAs that are highly structured require helicase activity for better translation efficiency. This process may need a scaffolding protein, like AKAP8L, in order to proceed.

Another interesting similarity between SKAR and AKAP8L is the potential of being a substrate for kinases in the mTOR pathway. It has already been demonstrated that SKAR is a substrate of S6K1 (68). However, AKAP8L has never been implicated as a substrate of S6K or kinases in the mTOR signaling pathway. In efforts to find new mTORC1 substrates and expand the phosphoproteome, two mass spectrometry studies identified a multitude of potential new targets (70,71). In those studies, the AKAP8L homolog AKAP8, was among those newly identified proteins with possible mTORC1 phosphorylation sites. Interestingly, when comparing sequences between AKAP8 and AKAP8L, there were conserved potential sites that could be targeted by mTORC1 for phosphorylation. A follow-up study indeed showed possible sites on AKAP8L, similar to our prediction using the AKAP8 sites (72). AKAP8L sites Ser 297, Ser 300 and Ser 302 of note, are located proximal to the nuclear export sequence (NES). It would be interesting if AKAP8L itself was a substrate of mTORC1. For example, AKAP8L phosphorylation near the NES by mTORC1 could regulate the subcellular localization and function of AKAP8L.

AKAP8L and AKAP8 share 61% protein sequence identity (46), prompting the question of how AKAP8L is able to interact with mTORC1 while AKAP8 is not (Fig. 1C-D). Within the AKAP8L region that interacts with mTORC1, amino acid 63-247, there is about 30% protein sequence similarity to AKAP8, possibly indicating the mTORC1 binding motif is not conserved. Alternatively, the nuclear export sequence (NES) motif on AKAP8L allows for the proper subcellular location in order to interact with mTORC1 (Fig. 1E). Another difference between AKAP8L and AKAP8 is the ability to bind R1α (46). Surprisingly, AKAP8L was observed to bind R1α (Fig. 3E-F), which has been shown to be more cytoplasmic (30-37). Additionally, R1α has previously been implicated in regulating mTORC1 mediated processes such as autophagy (73). Previously, AKAP8L was not considered a canonical AKAP due to the inability to bind
AKAP8L mediates protein translation

RIIα (46), however it is now more recognized that AKAP family members can bind RIIα/RIIβ or both (40). Because AKAP8L is not involved in the phosphorylation of Raptor at Ser791 by PKA (Supplemental Figure 3A), it suggests that AKAP8L has a different role in promoting mTORC1 mediated processes. Like AKAP1, which also associates with mTORC1 (44), AKAP8L positively mediates mTORC1 biology, and anchors the PKA holoenzyme. We currently have no evidence that PKA plays a direct role in respect to AKAP8L mediated mTORC1 biology. Activation of cAMP shows further decrease in cell proliferation in AKAP8L KO cells (Supplemental Fig. 9), indicating that cAMP signaling may regulate protein translation through another pathway (74).

Experimental Procedures

Cell lines and tissue culture - Human Embryonic Kidney 293A (HEK293A) cells were maintained at 37°C with 5% CO₂, cultured in high-glucose DMEM (#D5796 from Sigma) supplemented with 10% FBS (#F2442 from Sigma) and penicillin/streptomycin (#P0781 from Sigma, 100 units penicillin and 100 µg streptomycin/mL). For the generation of AKAP8L KO HEK293A cells stably expressing Flag-tagged full length AKAP8L or amino acid region 247-646, a lentiviral vector (addgene, #52962) encoding Flag-AKAP8L full length or Flag-AKAP8L amino acid region 247-646 was transfected in HEK293A cells with packaging plasmids (addgene, #12259 #12260), and the produced virus was collected from the medium 48 hrs after transfection. AKAP8L KO HEK293A cells were then infected with the lentivirus followed by selection of infected cells using blasticidin (5µg/ml; Life technologies REF A11139-03).

Antibodies - The following antibodies were purchased from Cell Signaling and used at the indicated dilution for western blot analysis: S6K (#9202, 1:1000), phospho-S6K (#9234, 1:1000), 4EBP1 (#9452, 1:1500), Raptor (#2280, 1:1000), mLST8 (#3274, 1:1000), DEPTOR (#11816, 1:1000), PRAS40 (#2691, 1:1000), phospho-Crebin (#9198S, 1:1000), Crebin (#9197S, 1:1000), Actin (#3700, 1:1000), Flag (#2044, 1:1000), Myc (#2276, 1:2000), phospho-PKA Substrate RRXS*/*T* (#9624, 1:1000), phospho-Akt Substrate RRXS*/*T* (#9614, 1:1000), Lamin A/C (#2032, 1:1000), Tubulin (#2144, 1:1000). Flag (#F1804, 1:5000) was obtained from Sigma. HA (#sc-7392, 1:500) was from Santa Cruz Biotechnology. mCherry (#GTX128508, 1:1000) and AKAP8L (#GTX115831, 1:1000) was from GeneTex. LARP1 (#A302-087A, 1:1000) and AKAP8 (#A301-061A, 1:1000) was from Bethyl Laboratories. Horseradish peroxidase (HRP) linked secondary antibodies (#NXA931V anti-mouse, 1:8000 or #NA934V anti-rabbit, 1:4000) were from GE Healthcare.

Plasmids - AKAP8L cDNA was cloned into a modified pcDNA based vector (pCCF) with an N-terminal Flag tag. Flag-tagged AKAP8L truncations were based off domains previously described (49). HA-RHA was obtained from Sino Biological (#HG17921-NY).

All data are contained within the manuscript. Detailed procedures are available in supporting information.

Acknowledgments: We thank members of the Buszczak and Mendell lab for their help with polysome profiling. We are grateful to all members of the Jewell laboratory for insightful discussions. We thank Noemi Eckhoff and Greg Urquhart for technical help. This work was supported by grants from Cancer Prevention Research Institute of Texas (CPRIT) Scholar Recruitment of First-Time, Tenure-Track Faculty Member (RR150032), Cancer Prevention Research Institute of Texas (CPRIT) High-Impact/High-Risk Research Award (RP160713), The Welch Foundation (I-1927-20170325), 2017 UT Southwestern President’s Research Council Distinguished Researcher Award, American Cancer Society Institutional Research Grant (ACS-IRG-17-174-13), and National Institutes of Health (R01GM129097-01) to J.L.J.

Author contributions: C.H.M and J.L.J designed the experiments. C.H.M and D.M. conducted the experiments. C.H.M and J.L.J wrote the manuscript.

Conflict of interest: The authors declare no conflicts of interest.
References

1. Ma, X. M., and Blenis, J. (2009) Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol 10, 307-318
2. Menon, S., and Manning, B. D. (2008) Common corruption of the mTOR signaling network in human tumors. Oncogene 27 Suppl 2, S43-51
3. Laplante, M., and Sabatini, D. M. (2012) mTOR signaling in growth control and disease. Cell 149, 274-293
4. Saxton, R. A., and Sabatini, D. M. (2017) mTOR Signaling in Growth, Metabolism, and Disease. Cell 169, 361-371
5. Sancak, Y., Bar-Peled, L., Zoncu, R., Markhard, A. L., Nada, S., and Sabatini, D. M. (2010) Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 141, 290-303
6. Sancak, Y., Peterson, T. R., Shaul, Y. D., Lindquist, R. A., Thoreen, C. C., Bar-Peled, L., and Sabatini, D. M. (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 320, 1496-1501
7. Jewell, J. L., Russell, R. C., and Guan, K. L. (2013) Amino acid signalling upstream of mTOR. Nat Rev Mol Cell Biol 14, 133-139
8. Jewell, J. L., and Guan, K. L. (2013) Nutrient signaling to mTOR and cell growth. Trends Biochem Sci 38, 233-242
9. Nandagopal, N., and Roux, P. P. (2015) Regulation of global and specific mRNA translation by the mTOR signaling pathway. Translation (Austin) 3, e983402
10. Foster, K. G., and Fingar, D. C. (2010) Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. J Biol Chem 285, 14071-14077
11. Roux, P. P., and Topisirovic, I. (2012) Regulation of mRNA translation by signaling pathways. Cold Spring Harb Perspect Biol 4
12. Hay, N., and Sonenberg, N. (2004) Upstream and downstream of mTOR. Genes Dev 18, 1926-1945
13. Dowling, R. J., Topisirovic, I., Alain, T., Bidinosti, M., Fonseca, B. D., Petroulakis, E., Wang, X., Larsson, O., Selvaraj, A., Liu, Y., Kozma, S. C., Thomas, G., and Sonenberg, N. (2010) mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. Science 328, 1172-1176
14. Lin, T. A., Kong, X., Haystead, T. A., Pause, A., Belsham, G., Sonenberg, N., and Lawrence, J. C., Jr. (1994) PHAS-I as a link between mitogen-activated protein kinase and translation initiation. Science 266, 653-656
15. Pause, A., Belsham, G. J., Gingras, A. C., Donze, O., Lin, T. A., Lawrence, J. C., Jr., and Sonenberg, N. (1994) Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. Nature 371, 762-767
16. Hsieh, A. C., Liu, Y., Edlind, M. P., Ingolia, N. T., Janes, M. R., Sher, A., Shi, E. Y., Stumpf, C. R., Christensen, C., Bonham, M. J., Wang, S., Ren, P., Martin, M., Jessen, K., Feldman, M. E., Weissman, J. S., Shokat, K. M., Rommel, C., and Ruggero, D. (2012) The translational landscape of mTOR signalling steers cancer initiation and metastasis. Nature 485, 55-61
17. Thoreen, C. C., Chantranupong, L., Keys, H. R., Wang, T., Gray, N. S., and Sabatini, D. M. (2012) A unifying model for mTORC1-mediated regulation of mRNA translation. Nature 485, 109-113
18. Jefferies, H. B., Reinhard, C., Kozma, S. C., and Thomas, G. (1994) Rapamycin selectively represses translation of the "polypyrimidine tract" mRNA family. *Proc Natl Acad Sci U S A* **91**, 4441-4445

19. Hong, S., Freeberg, M. A., Han, T., Kamath, A., Yao, Y., Fukuda, T., Suzuki, T., Kim, J. K., and Inoki, K. (2017) LARP1 functions as a molecular switch for mTORC1-mediated translation of an essential class of mRNAs. *Elife* **6**

20. Tcherkezian, J., Cargnello, M., Romeo, Y., Huttlin, E. L., Lavoie, G., Gygi, S. P., and Roux, P. P. (2014) Proteomic analysis of cap-dependent translation identifies LARP1 as a key regulator of 5'TOP mRNA translation. *Genes Dev* **28**, 357-371

21. Fonseca, B. D., Zakaria, C., Jia, J. J., Graber, T. E., Svitkin, Y., Tahmasebi, S., Healy, D., Hoang, H. D., Jensen, J. M., Diao, I. T., Lussier, A., Dajadian, C., Padmanabhan, N., Wang, W., Matta-Camacho, E., Hearnden, J., Smith, E. M., Tsukumo, Y., Yanagiyama, A., Morita, M., Petroulakis, E., Gonzalez, J. L., Hernandez, G., Alain, T., and Damgaard, C. K. (2015) La-related Protein 1 (LARP1) Represses Terminal Oligopyrimidine (TOP) mRNA Translation Downstream of mTOR Complex 1 (mTORC1). *J Biol Chem* **290**, 15996-16020

22. Philippe, L., Vasseur, J. J., Debar t, F., and Thoreen, C. C. (2018) La-related protein 1 (LARP1) repression of TOP mRNA translation is mediated through its cap-binding domain and controlled by an adjacent regulatory region. *Nucleic Acids Res* **46**, 1457-1469

23. Egan, D., Kim, J., Shaw, R. J., and Guan, K. L. (2011) The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. *Autophagy* **7**, 643-644

24. Hauser, A. S., Attwood, M. M., Rask-Andersen, M., Schioth, H. B., and Gloriam, D. E. (2017) Trends in GPCR drug discovery: new agents, targets and indications. *Nat Rev Drug Discov* **16**, 829-842

25. Malbon, C. C., Tao, J., and Wang, H. Y. (2004) AKAPs (A-kinase anchoring proteins) and molecules that compose their G-protein-coupled receptor signalling complexes. *Biochem J* **379**, 1-9

26. Hanlon, C. D., and Andrew, D. J. (2015) Outside-in signaling--a brief review of GPCR signaling with a focus on the Drosophila GPCR family. *J Cell Sci* **128**, 3533-3542

27. Pavlos, N. J., and Friedman, P. A. (2017) GPCR Signaling and Trafficking: The Long and Short of It. *Trends Endocrinol Metab* **28**, 213-226

28. Francis, S. H., and Corbin, J. D. (1994) Structure and function of cyclic nucleotide-dependent protein kinases. *Annu Rev Physiol* **56**, 237-272

29. Knighton, D. R., Zheng, J. H., Ten Eyck, L. F., Ashford, V. A., Xuong, N. H., Taylor, S. S., and Sowadski, J. M. (1991) Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. *Science* **253**, 407-414

30. Rubin, C. S., Erlichman, J., and Rosen, O. M. (1972) Cyclic adenosine 3',5'-monophosphate-dependent protein kinase of human erythrocyte membranes. *J Biol Chem* **247**, 6135-6139
AKAP8L mediates protein translation

31. Sarkar, D., Erlichman, J., and Rubin, C. S. (1984) Identification of a calmodulin-binding protein that co-purifies with the regulatory subunit of brain protein kinase II. J Biol Chem 259, 9840-9846
32. Nigg, E. A., Schafer, G., Hilz, H., and Eppenberger, H. M. (1985) Cyclic-AMP-dependent protein kinase type II is associated with the Golgi complex and with centrosomes. Cell 41, 1039-1051
33. Cadd, G., and McKnight, G. S. (1989) Distinct patterns of cAMP-dependent protein kinase gene expression in mouse brain. Neuron 3, 71-79
34. Salvatori, S., Damiani, E., Barhanin, J., Furlan, S., Salviati, G., and Margreth, A. (1990) Co-localization of the dihydropyridine receptor and the cyclic AMP-binding subunit of an intrinsic protein kinase to the junctional membrane of the transverse tubules of skeletal muscle. Biochem J 267, 679-687
35. Joachim, S., and Schwoch, G. (1990) Localization of cAMP-dependent protein kinase subunits along the secretory pathway in pancreatic and parotid acinar cells and accumulation of the catalytic subunit in parotid secretory granules following beta-adrenergic stimulation. Eur J Cell Biol 51, 76-84
36. Scott, J. D. (1991) Cyclic nucleotide-dependent protein kinases. Pharmacol Ther 50, 123-145
37. Doskeland, S. O., Maronde, E., and Gjertsen, B. T. (1993) The genetic subtypes of cAMP-dependent protein kinase--functionally different or redundant? Biochim Biophys Acta 1178, 249-258
38. Jahnsen, T., Hedin, L., Lohmann, S. M., Walter, U., and Richards, J. S. (1986) The neural type II regulatory subunit of cAMP-dependent protein kinase is present and regulated by hormones in the rat ovary. J Biol Chem 261, 6637-6639
39. Delghandi, M. P., Johannessen, M., and Moens, U. (2005) The cAMP signalling pathway activates CREB through PKA, p38 and MSK1 in NIH 3T3 cells. Cell Signal 17, 1343-1351
40. Wong, W., and Scott, J. D. (2004) AKAP signalling complexes: focal points in space and time. Nat Rev Mol Cell Biol 5, 959-970
41. Kapiloff, M. S., Rigatti, M., and Dodge-Kafka, K. L. (2014) Architectural and functional roles of A kinase-anchoring proteins in cAMP microdomains. J Gen Physiol 143, 9-15
42. Esseltine, J. L., and Scott, J. D. (2013) AKAP signaling complexes: pointing towards the next generation of therapeutic targets? Trends Pharmacol Sci 34, 648-655
43. Perez Lopez, I., Cariolato, L., Maric, D., Gillet, L., Abriel, H., and Diviani, D. (2013) A-kinase anchoring protein Lbc coordinates a p38 activating signaling complex controlling compensatory cardiac hypertrophy. Mol Cell Biol 33, 2903-2917
44. Rinaldi, L., Sepe, M., Delle Donne, R., Conte, K., Arcella, A., Borzacchiello, D., Amente, S., De Vita, F., Porpora, M., Garbi, C., Oliva, M. A., Procaccini, C., Faicchia, D., Matarese, G., Zito Marino, F., Rocco, G., Pignatiello, S., Franco, R., Insabato, L., Majello, B., and Feliciello, A. (2017) Mitochondrial AKAP1 supports mTOR pathway and tumor growth. Cell Death Dis 8, e2842
45. Jewell, J. L., Fu, V., Hong, A. W., Yu, F. X., Meng, D., Melick, C. H., Wang, H., Lam, W. M., Yuan, H. X., Taylor, S. S., and Guan, K. L. (2019) GPCR signaling inhibits mTORC1 via PKA phosphorylation of Raptor. *Elife* 8

46. Orstavik, S., Eide, T., Collas, P., Han, I. O., Tasken, K., Kieff, E., Jahnsen, T., and Skalhegg, B. S. (2000) Identification, cloning and characterization of a novel nuclear protein, HA95, homologous to A-kinase anchoring protein 95. *Biol Cell* 92, 27-37

47. Ryan, K. J., and Wente, S. R. (2000) The nuclear pore complex: a protein machine bridging the nucleus and cytoplasm. *Curr Opin Cell Biol* 12, 361-371

48. Koyama, S., Yu, H., Dalgarno, D. C., Shin, T. B., Zydowsky, L. D., and Schreiber, S. L. (1993) Structure of the PI3K SH3 domain and analysis of the SH3 family. *Cell* 72, 945-952

49. Yang, J. P., Tang, H., Reddy, T. R., and Wong-Staal, F. (2001) Mapping the functional domains of HAP95, a protein that binds RNA helicase A and activates the constitutive transport element of type D retroviruses. *J Biol Chem* 276, 30694-30700

50. Martins, S. B., Eide, T., Steen, R. L., Jahnsen, T., Skalhegg, B. S., and Collas, P. (2000) HA95 is a protein of the chromatin and nuclear matrix regulating nuclear envelope dynamics. *J Cell Sci* 113 Pt 21, 3703-3713

51. Martins, S., Eikvar, S., Furukawa, K., and Collas, P. (2003) HA95 and LAP2 beta mediate a novel chromatin-nuclear envelope interaction implicated in initiation of DNA replication. *J Cell Biol* 160, 177-188

52. Westberg, C., Yang, J. P., Tang, H., Reddy, T. R., and Wong-Staal, F. (2000) A novel shuttle protein binds to RNA helicase A and activates the retroviral constitutive transport element. *J Biol Chem* 275, 21396-21401

53. Coghlan, V. M., Langeberg, L. K., Fernandez, A., Lamb, N. J., and Scott, J. D. (1994) Cloning and characterization of AKAP 95, a nuclear protein that associates with the regulatory subunit of type II cAMP-dependent protein kinase. *J Biol Chem* 269, 7658-7665

54. Eide, T., Coghlan, V., Orstavik, S., Holseve, C., Solberg, R., Skalhegg, B. S., Lamb, N. J., Langeberg, L., Fernandez, A., Scott, J. D., Jahnsen, T., and Tasken, K. (1998) Molecular cloning, chromosomal localization, and cell cycle-dependent subcellular distribution of the A-kinase anchoring protein, AKAP95. *Exp Cell Res* 238, 305-316

55. Zhu, X., Zelmer, A., and Wellmann, S. (2017) Visualization of Protein-protein Interaction in Nuclear and Cytoplasmic Fractions by Co-immunoprecipitation and In Situ Proximity Ligation Assay. *J Vis Exp*

56. Baroni, M. D., Martegani, E., Monti, P., and Alberghina, L. (1989) Cell size modulation by CDC25 and RAS2 genes in Saccharomyces cerevisiae. *Mol Cell Biol* 9, 2715-2723

57. Tokiwa, G., Tyers, M., Volpe, T., and Futcher, B. (1994) Inhibition of G1 cyclin activity by the Ras/cAMP pathway in yeast. *Nature* 371, 342-345

58. Gutzkow, K. B., Lahne, H. U., Naderi, S., Torgersen, K. M., Skalhegg, B., Koketsu, M., Uehara, Y., and Blomhoff, H. K. (2003) Cyclic AMP inhibits translation of cyclin D3 in T lymphocytes at the level of elongation by inducing eEF2-phosphorylation. *Cell Signal* 15, 871-881
59. Han, I., Xue, Y., Harada, S., Orstavik, S., Skalhegg, B., and Kieff, E. (2002) Protein kinase A associates with HA95 and affects transcriptional coactivation by Epstein-Barr virus nuclear proteins. *Mol Cell Biol* **22**, 2136-2146

60. Hartman, T. R., Qian, S., Bolinger, C., Fernandez, S., Schoenberg, D. R., and Boris-Lawrie, K. (2006) RNA helicase A is necessary for translation of selected messenger RNAs. *Nat Struct Mol Biol* **13**, 509-516

61. Parsyan, A., Svitkin, Y., Shahbazian, D., Gkogkas, C., Lasko, P., Merrick, W. C., and Sonenberg, N. (2011) mRNA helicases: the tacticians of translational control. *Nat Rev Mol Cell Biol* **12**, 235-245

62. Avni, D., Biberman, Y., and Meyuhas, O. (1997) The 5' terminal oligopyrimidine tract confers translational control on TOP mRNAs in a cell type- and sequence context-dependent manner. *Nucleic Acids Res* **25**, 995-1001

63. Fingar, D. C., Salama, S., Tsou, C., Harlow, E., and Blenis, J. (2002) Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes Dev* **16**, 1472-1487

64. Chung, J., Kuo, C. J., Crabtree, G. R., and Blenis, J. (1992) Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. *Cell* **69**, 1227-1236

65. Barbet, N. C., Schneider, U., Helliwell, S. B., Stansfield, I., Tuite, M. F., and Hall, M. N. (1996) TOR controls translation initiation and early G1 progression in yeast. *Mol Biol Cell* **7**, 25-42

66. Beretta, L., Gingras, A. C., Svitkin, Y. V., Hall, M. N., and Sonenberg, N. (1996) Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. *EMBO J* **15**, 658-664

67. Jefferies, H. B., Fumagalli, S., Dennis, P. B., Reinhard, C., Pearson, R. B., and Thomas, G. (1997) Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70s6k. *EMBO J* **16**, 3693-3704

68. Richardson, C. J., Broenstrup, M., Fingar, D. C., Julich, K., Ballif, B. A., Gygi, S., and Blenis, J. (2004) SKAR is a specific target of S6 kinase 1 in cell growth control. *Curr Biol* **14**, 1540-1549

69. Ma, X. M., Yoon, S. O., Richardson, C. J., Julich, K., and Blenis, J. (2008) SKAR links pre-mRNA splicing to mTOR/S6K1-mediated enhanced translation efficiency of spliced mRNAs. *Cell* **133**, 303-313

70. Hsu, P. P., Kang, S. A., Rameseder, J., Zhang, Y., Ottina, K. A., Lim, D., Peterson, T. R., Choi, Y., Gray, N. S., Yaffe, M. B., Marto, J. A., and Sabatini, D. M. (2011) The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. *Science* **332**, 1317-1322

71. Yu, Y., Yoon, S. O., Poulougliannis, G., Yang, Q., Ma, X. M., Villen, J., Kubica, N., Hoffman, G. R., Cantley, L. C., Gygi, S. P., and Blenis, J. (2011) Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. *Science* **332**, 1322-1326

72. Zhang, Y., Zhang, Y., and Yu, Y. (2017) Global Phosphoproteomic Analysis of Insulin/Akt/mTORC1/S6K Signaling in Rat Hepatocytes. *J Proteome Res* **16**, 2825-2835

73. Mavrakis, M., Lippincott-Schwartz, J., Stratakis, C. A., and Bossis, I. (2006) Depletion of type IA regulatory subunit (R1alpha) of protein kinase A (PKA) in
mammalian cells and tissues activates mTOR and causes autophagic deficiency. 
*Hum Mol Genet* **15**, 2962-2971

Yu, F. X., Zhang, Y., Park, H. W., Jewell, J. L., Chen, Q., Deng, Y., Pan, D., 
Taylor, S. S., Lai, Z. C., and Guan, K. L. (2013) Protein kinase A activates the 
Hippo pathway to modulate cell proliferation and differentiation. *Genes Dev* **27**, 
1223-1232
Figure 1. AKAP8L is an mTORC1 interacting protein. (A) Raptor interacts with AKAP8L. Co-immunoprecipitation of Flag-tagged AKAP8L with HA-tagged Raptor. (B) Co-immunoprecipitation of HA-tagged Raptor with Flag-tagged AKAP8L. (C) Co-immunoprecipitation of endogenous AKAP8L with endogenous Raptor. (D) Co-immunoprecipitation of HA-tagged Raptor with endogenous AKAP8L. (E) Schematic of the domains of AKAP8L and AKAP8. YG = YG rich domain, FG = FG repeat region, NLS = nuclear localization sequence, NES = nuclear export sequence. (F) mTORC1 interacts with AKAP8L. Co-immunoprecipitation of HA-tagged Raptor and endogenous mTORC1 components with Flag-tagged AKAP8L. IP = immunoprecipitation. WCL = Whole cell lysate. EV = Empty vector.
Figure 2. The N-terminus of AKAP8L is required to interact with mTORC1. (A) Schematic of AKAP8L truncations generated based off relevant domains. (B) AKAP8L amino acid region 247-646 does not interact with Raptor. Co-immunoprecipitation of HA-tagged Raptor with indicated Flag-tagged AKAP8L truncations. (C) AKAP8L amino acid region 63-247 interacts with Raptor. Co-immunoprecipitation of Flag-tagged AKAP8L amino acid region 63-247 with HA-tagged Raptor. IP = immunoprecipitation. WCL = Whole cell lysate. FL = Full length. RFP = Red fluorescent protein.
Figure 3. AKAP8L interacts with mTORC1 in the cytoplasm. (A) AKAP8L localizes to the cytoplasm and nucleus. Expression of Flag-tagged AKAP8L in mouse embryonic fibroblast (MEF) cells. Red denotes Flag-tagged AKAP8L (1.0 µg). (B) Expression of Flag-tagged AKAP8L in the cytoplasmic and nuclear fractions of HEK293A cells. (C) Expression of endogenous AKAP8L in the cytoplasmic and nuclear fractions of HEK293A cells. (D) Raptor interacts with AKAP8L in the cytoplasm. Co-immunoprecipitation of endogenous AKAP8L with HA-tagged Raptor. (E) AKAP8L interacts with Rlα. Co-immunoprecipitation of mCherry-tagged Rlα with Flag-tagged AKAP8L. (F) Raptor interacts with AKAP8L and PKA machinery. Co-immunoprecipitation of Flag-tagged AKAP8L, mCherry-tagged Rlα and Flag-tagged PKA Catα with HA-tagged Raptor. IP = immunoprecipitation. WCL = Whole cell lysate. s.e = short exposure. l.e. = long exposure.
AKAP8L mediates protein translation

A) Representative Polyribosome Profile

B) Representative Polysome Profile

C) Representative Cell Size

D) Cell Proliferation

E) mTORC1

- AKAP8L
- SGK
- 4EBP1
- LAAP1
- ?

Translation
Cell Size
Cell Proliferation

- sgGFP + Flag-EV
- sgAKAP8L#1 + Flag EV
- sgAKAP8L#1 + Flag-AKAP8L
- sgAKAP8L#1 + Flag-AKAP8L 247-640
**Figure 4. AKAP8L regulates protein translation, cell size and proliferation.** (A) Depletion of AKAP8L decreases global protein synthesis. AKAP8L knockout (KO) cells were incubated in methionine and cysteine-free DMEM for 1 hr. Cycloheximide (CHX) treatment for 1 hr was used as a positive control. 35S-labeled L-methionine and L-cysteine mix was then added to the medium for 10 mins and newly synthesized proteins were detected by autoradiography. Values displayed as means ± SD. Significance was analyzed using Student’s T-test. (B) Loss of AKAP8L reduces number of polysomes. AKAP8L KO cells with or without stably expressing full length Flag-tagged AKAP8L were subjected to polysome profiling. Negative control WT cells were treated with 100nM Torin1 for 1 hr. Representative image shown from 3 biological replicates. (C) Loss of AKAP8L reduces cell size. The size of AKAP8L KO cells with or without stably expressing full length Flag-tagged AKAP8L or Flag-tagged AKAP8L 63-247 were measured using a coulter counter. Samples with closest value to mean plotted as a representative image. Significance \( (P) \): sgGFP vs. sgAKAP8L#1 + FlagEV = < 0.01, sgGFP vs. FlagAKAP8L 247-646 = < 0.01, sgAKAP8L#1 + FlagAKAP8L vs. sgAKAP8L#1 + FlagEV = <0.001, sgAKAP8L#1 + FlagAKAP8L vs. sgAKAP8L#1 + FlagAKAP8L 247-646 = <0.001. Significance was analyzed using Student’s T-test. Number of biological repeats is \( n \geq 3 \). Number of technical repeats of each sample is \( n = 3 \) per experiment. (D) Loss of AKAP8L reduces cell proliferation. AKAP8L KO cells with or without stably expressing full length Flag-tagged AKAP8L or Flag-tagged AKAP8L 63-247 were counted on the indicated days using Trypan Blue and a BioRad automated cell counter. Values are displayed as means ± SD. Significance was analyzed using Student’s T-test. Number of biological repeats is \( n \geq 3 \). Number of technical repeats of each sample is \( n = 3 \) per experiment. (E) Working model of AKAP8L regulating translation, cell size and proliferation. Significance \( (P) \): * = < 0.05, ** = < 0.01, *** = < 0.001.
A-kinase anchoring protein 8L interacts with mTORC1 and promotes cell growth
Chase H. Melick, Delong Meng and Jenna L. Jewell

J. Biol. Chem. published online April 20, 2020

Access the most updated version of this article at doi: 10.1074/jbc.AC120.012595

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts