Adipocyte 1:4, 246–249; October/November/December 2012; © 2012 Landes Bioscience

Stay lean without dieting

Lose Sam68

Marc-Étienne Huot[1,*] and Stéphane Richard[2]

[1]Laval University Cancer Research Center; Hôtel-Dieu de Québec; CHUQ; Quebec City, QC Canada; [2]Terry Fox Molecular Oncology Group and Bloomfield Center for Research on Aging; Lady Davis Institute for Medical Research and Departments of Oncology and Medicine; McGill University; Montreal, QC Canada

Alternative splicing is well known to be tissue-specific. Although several genes have been shown to undergo alternative splicing in adipocytes, little is known about the mechanism that regulates alternative splicing during adipogenesis. We recently reported that Sam68$^{-/-}$ mice exhibit a lean phenotype and are protected against diet-induced obesity. Our genome-wide exon array analysis in white adipose tissue (WAT) from wild-type and Sam68$^{-/-}$ mice revealed that Sam68 deficiency leads to an abnormal splicing of the mTOR gene. This has been shown to reduce the overall mTOR protein content and activity during in vitro adipose differentiation. In Sam68$^{-/-}$ mice, this situation leads to an increased energy expenditure, decreased adipogenesis and WAT formation.

Keywords: alternative splicing, white adipose tissue, mTOR, Sam68, STAR proteins, adipose differentiation

Submitted: 04/04/12

Revised: 05/17/12

Accepted: 05/21/12

http://dx.doi.org/10.4161/adip.20819

*Correspondence to: Marc-Étienne Huot; Email: marc-etienne.huot@crchuq.ulaval.ca

Commentary to: Huot MÉ, Vogel G, Zabarauskas A, Ngo CT, Coulombe-Huntington J, Majewski J, et al. The Sam68 STAR RNA-Binding Protein Regulates mTOR Alternative Splicing during Adipogenesis. Mol Cell 2012; 46:187–99; PMID:22424772; http://dx.doi.org/10.1016/j.molcel.2012.02.007

Introduction

The **S**rc-**a**ssociated in **m**itosis 68 kDa protein (Sam68) is a member of the **s**ignal **t**ransduction and **a**ctivation of **R**NA (STAR) family of proteins.[1] The recent identification of Sam68-interacting proteins has shown that Sam68 is involved in several signal transduction pathways via its association with SH3- and SH2-containing signaling molecules, suggesting that Sam68 is an adaptor protein.[1-3] The trademark of all STAR proteins is the presence of a single hnRNPK homology (KH) domain known to bind RNA with a high relative affinity.[4] Sam68 has been shown to bind to a bipartite sequence of U(U/A)AA rich motifs.[5,6] Sam68 is a known regulator of alternative splicing. Sam68 has been shown to regulate the inclusion of the variable exon v5 of CD44,[7] a cell surface glycoprotein involved in tumor invasion.[7-9] Sam68 also modulates the splicing of mRNAs encoding SF2/ASF, SMN2, the proapoptotic protein Bcl-x, the cancer-related splice isoform cyclin D1b and the neurexin 1 gene.[10-13] More extensive studies have recently defined Sam68 as major regulator of neurogenesis,[10,14] spermatogenesis,[15] osteogenesis[16] and more recently, adipogenesis.[5] These studies clearly indicate that Sam68 regulates alternative splicing during cellular differentiation.

The availability of Sam68-deficient mice has led to the identification of some of the physiological roles of this KH-type RNA-binding protein. Sam68$^{-/-}$ mice do not display any overt phenotype, but male mice are infertile due to a spermatogenesis defect, while the females have reduced fertility due to defects in the proper expression of gonadotropin receptor transcripts in pre-ovulatory follicles in the adult ovary.[15-18] Moreover, Sam68$^{-/-}$ mice are protected against age-induced osteoporosis.[16] Indeed, aged Sam68$^{-/-}$ mice have been shown to preserve bone density via the Sam68-dependent promotion of osteoblast differentiation and are thus protected against age-related bone loss.[16] These results suggest that Sam68 is directly involved in mesenchymal stem cell differentiation. The loss of Sam68 expression shifts mesenchymal stem cell differentiation toward the osteogenic lineage, instead of the adipocytic lineage. This was confirmed by our recent demonstration that Sam68-deficient mice are lean and protected against diet-induced obesity.[5]

Sam68 and Adipogenesis

To confirm a role for Sam68 in the regulation of adipocyte differentiation, we initially characterized the primary MEFs harvested from Sam68$^{+/+}$ and Sam68$^{-/-}$ embryos. Adipogenesis was monitored at days 0, 4, 6 and 12 following initial stimulation with standard adipogenic differentiation media. As expected, we found that primary Sam68$^{-/-}$ MEFs were unable to differentiate into adipocytes, unlike their Sam68$^{+/+}$ counterparts. Magnetic resonance imaging studies of both Sam68$^{+/+}$ and Sam68$^{-/-}$ aging mice showed that Sam68 inactivation affects mouse weight independently of food consumption. We also determined the respiratory exchange ratio, which was found to be similar in Sam68$^{+/+}$ and Sam68$^{-/-}$, suggesting that both mouse genotypes primarily utilize fatty acids as an energy source. On the other hand, we have shown that Sam68$^{-/-}$ mice exhibit a higher energy expenditure than Sam68$^{+/+}$ mice, likely due to increased physical activity, which contributes to the lean phenotype. This difference in whole body weight was more striking when mice were fed a high-fat diet. The latter experiment clearly showed that Sam68$^{-/-}$ mice are unable to gain weight under a high-fat diet, whereas the wild-type littermates significantly increased in weight. In-depth analysis of adipose tissue revealed that Sam68$^{-/-}$ mice had a reduced number of adult-derived stem cells (i.e., adipogenic progenitors), correlating with a decrease in the expression of pericyte markers (α-SMA and NG2). Taken together, these results show that Sam68 is required for normal adipogenesis.

mTOR Splicing

Using a genome-wide exon expression array, we found that the loss of Sam68 influences the splicing of a large number of genes in white adipose tissue. We showed that the splicing of the mTOR gene is greatly influenced by the levels of Sam68. mTOR belongs to the phosphatidylinositol 3-kinase-related protein family and is known to regulate key cellular processes, such as cell size, cell proliferation, cell motility and cell survival.[19] mTOR is the catalytic subunit found in two different complexes (mTORC1 and mTORC2).[20] mTORC1 is characterized by its association with the protein Raptor and its sensitivity toward rapamycin, whereas mTORC2 is characterized by the presence of the protein Rictor and its resistance to rapamycin.[19] The inhibition of mTORC1 is known to prevent the adipogenic differentiation of pre-adipocytes,[21,22] and to downregulate fat deposition in rodents.[23,24] Moreover, the inhibition of mTORC1 signaling in mice leads to lean phenotypes.[25-27]

For the above-mentioned reasons, we decided to investigate the Sam68-dependent alternative splicing of mTOR in greater detail.[5] We found that the loss of Sam68 activates the usage of an intronic polyadenylation signal within intron 5 of the mTOR gene. This novel isoform that we designate as mTOR$_{i5}$, includes exons 1 to 5 plus a readthrough of intron 5, thereby generating a ~1 kb mRNA (**Fig. 1A**). We showed that Sam68 associates with two short UUUUA sequences within intron 5. Mutations within each of the two UUUUA elements of the sequences influence Sam68 binding and lead to an increase in mTORi5 isoform protein level.

mTOR$_{i5}$

Using qRT-PCR, we observed that mTOR$_{i5}$ mRNA is abundant in Sam68-deficient 3T3-L1 cells but absent in control 3T3-L1 cells. As mTOR intron 5 harbors an in-frame premature termination codon as well as a polyadenylation signal, the intron retention event is expected to lead to an mTOR protein of ~25 kDa with a large C-terminal truncation. The protein predicted to be expressed from the mTOR$_{i5}$ mRNA would contain most of the N-terminal HEAT domains, a type of protein-protein interaction domain found in a number of cytoplasmic proteins.[28] This led us to believe that the mTOR$_{i5}$ protein might exhibit dominant-negative behavior via its binding to a component of the mTORC1/2 complexes, thus forming a catalytically inactive complex. To assess this possibility, we introduced a vector that expresses a FLAG-tagged mTOR$_{i5}$ protein in HeLa cells. We then monitored the activity of the mTORC1/2 complexes, using ribosomal protein S6 phospho-specific antibodies (to detect mTORC1 activity) and AKT phospho-specific antibodies (to assess mTORC2 activity). Although the ~25 kDa FLAG-mTOR$_{i5}$ protein was effectively expressed in these cells, we found that it had virtually no influence on the levels of either ribosomal protein S6 S240/S244 or AKT S473 phosphorylation following insulin stimulation. Moreover, we were unable to detect the mTOR$_{i5}$ protein in cells in which Sam68 expression was abrogated using N-terminal-specific commercial antibodies that recognized the ectopic expression of FLAG-mTOR$_{i5}$. These results allowed us to rule out any dominant-negative function for the mTOR$_{i5}$ protein and suggested that the mTOR$_{i5}$ mRNA or protein might be rapidly degraded via an as yet unidentified mechanism.

Sam68 Regulates mTOR Activity

One major effect of generating the mTOR$_{i5}$ isoform is a decrease in mTOR mRNA and protein levels. Since, mTORC1 is essential for normal adipogenesis, and as depletion of the major component of the complex, namely mTOR, affects pre-adipocyte differentiation, a deficiency in Sam68 expression leads to a decrease in mTORC1 activity. The overall effect of reduced mTORC1 activity is an inhibition of pre-adipocyte differentiation (**Fig. 1B**). Interestingly, we were able to pre-empt the latter phenotype by reintroducing full-length mTOR in Sam68-deficient 3T3-L1 cells. The latter result indicates that Sam68 contributes in adipogenesis by insuring the proper splicing of intron 5 in the course of mTOR mRNA synthesis, thus allowing the expression of full-length mTOR.

Conclusion

Our work suggests that Sam68 is a key regulator of alternative splicing in white adipose tissue. Our results offer new therapeutic opportunities for blocking adipogenesis and diet-induced obesity, which could prevent the long-term complications related to diet-induced obesity, one of the leading preventable causes of death in North America.

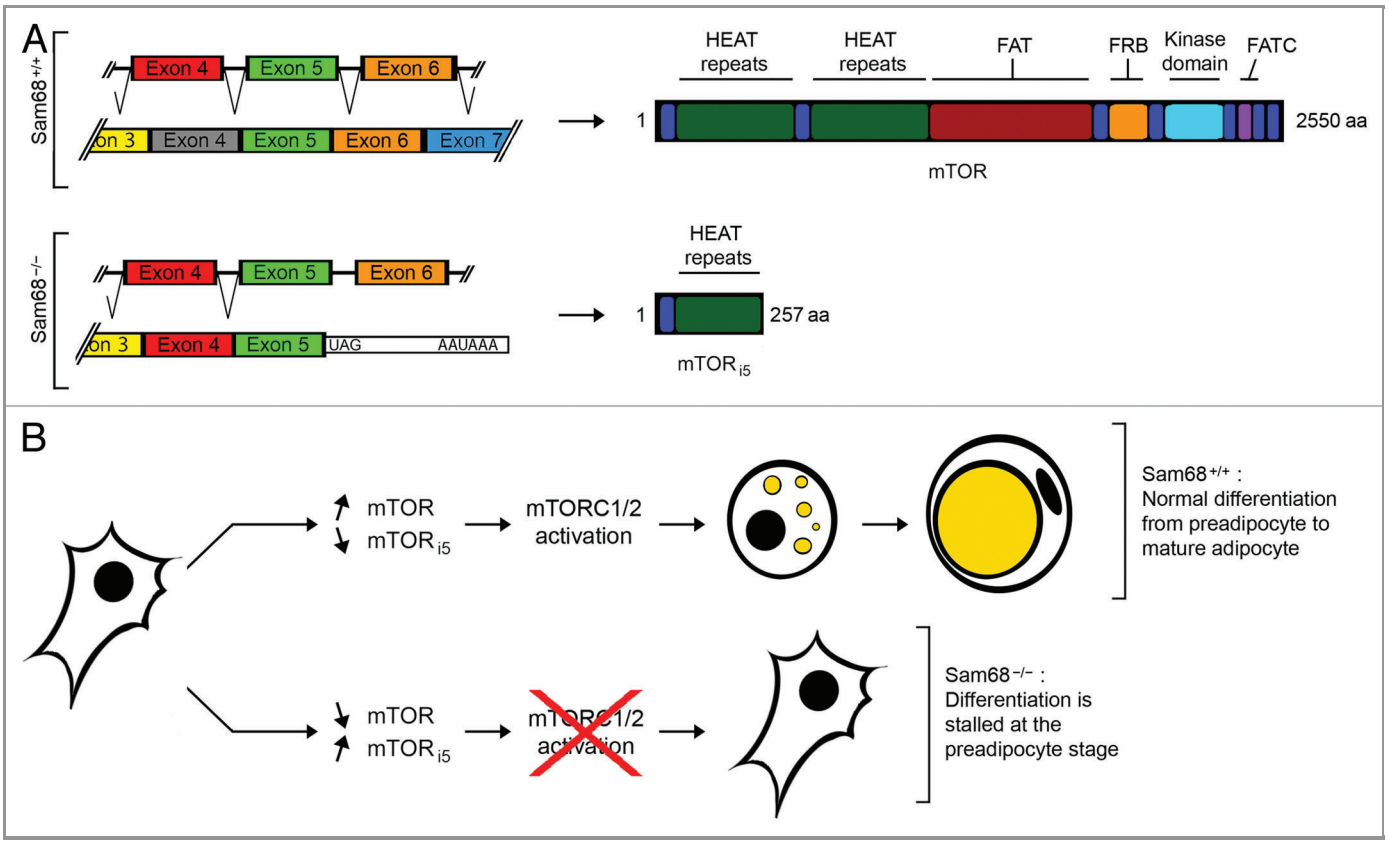

Figure 1. Schematic representation of the function of Sam68 during adipogenesis. (A) Effect of Sam68 on mTOR mRNA splicing. Sam68 inactivation or impairing its binding to specific elements present in mTOR intron 5 increases read-through and inclusion of intron 5, thereby activating an intronic polyadenylation signal. This leads to a novel variant of mTOR mRNA whose translation predicts the synthesis of a truncated version of mTOR, termed $mTOR_{i5}$. (B) Sam68 inactivation blocks the progression of adipogenesis at the preadipocyte level, due to a decreased pool of full-length mTOR protein, which results in the lack of mTORC1/2 activation.

References

1. Lukong KE, Richard S. Sam68, the KH domain-containing superSTAR. Biochim Biophys Acta 2003; 1653:73-86; PMID:14643926
2. Huot ME, Brown CM, Lamarche-Vane N, Richard S. An adaptor role for cytoplasmic Sam68 in modulating Src activity during cell polarization. Mol Cell Biol 2009; 29:1933-43; PMID:19139276; http://dx.doi.org/10.1128/MCB.01707-08
3. Richard S, Yu D, Blumer KJ, Hausladen D, Olszowy MW, Connelly PA, et al. Association of p62, a multifunctional SH2- and SH3-domain-binding protein, with src family tyrosine kinases, Grb2, and phospholipase C gamma-1. Mol Cell Biol 1995; 15:186-97; PMID:7799925
4. Chen T, Damaj BB, Herrera C, Lasko P, Richard S. Self-association of the single-KH-domain family members Sam68, GRP33, GLD-1, and Qk1: role of the KH domain. Mol Cell Biol 1997; 17:5707-18; PMID:9315629
5. Huot ME, Vogel G, Zabarauskas A, Ngo CT, Coulombe-Huntington J, Majewski J, et al. The Sam68 STAR RNA-Binding Protein Regulates mTOR Alternative Splicing during Adipogenesis. Mol Cell 2012; 46:187-99; PMID:22424772; http://dx.doi.org/10.1016/j.molcel.2012.02.007
6. Lin Q, Taylor SJ, Shalloway D. Specificity and determinants of Sam68 RNA binding. Implications for the biological function of K homology domains. J Biol Chem 1997; 272:27274-80; PMID:9341174; http://dx.doi.org/10.1074/jbc.272.43.27274
7. Matter N, Herrlich P, König H. Signal-dependent regulation of splicing via phosphorylation of Sam68. Nature 2002; 420:691-5; PMID:12478298; http://dx.doi.org/10.1038/nature01153
8. Ponta H, Sherman L, Herrlich PA. CD44: from adhesion molecules to signalling regulators. Nat Rev Mol Cell Biol 2003; 4:33-45; PMID:12511867; http://dx.doi.org/10.1038/nrm1004
9. Batsché E, Yaniv M, Muchardt C. The human SWI/SNF subunit Brm is a regulator of alternative splicing. Nat Struct Mol Biol 2006; 13:22-9; PMID:16341228; http://dx.doi.org/10.1038/nsmb1030
10. Iijima T, Wu K, Witte H, Hanno-Iijima Y, Glatter T, Richard S, et al. SAM68 regulates neuronal activity-dependent alternative splicing of neurexin-1. Cell 2011; 147:1601-14; PMID:22196734; http://dx.doi.org/10.1016/j.cell.2011.11.028
11. Paronetto MP, Achsel T, Massiello A, Chalfant CE, Sette C. The RNA-binding protein Sam68 modulates the alternative splicing of Bcl-x. J Cell Biol 2007; 176:929-39; PMID:17371836; http://dx.doi.org/10.1083/jcb.200701005
12. Paronetto MP, Cappellari M, Busà R, Pedrotti S, Vitali R, Comstock C, et al. Alternative splicing of the cyclin D1 proto-oncogene is regulated by the RNA-binding protein Sam68. Cancer Res 2010; 70:229-39; PMID:20028857; http://dx.doi.org/10.1158/0008-5472.CAN-09-2788
13. Valacca C, Bonomi S, Buratti E, Pedrotti S, Baralle FE, Sette C, et al. Sam68 regulates EMT through alternative splicing-activated nonsense-mediated mRNA decay of the SF2/ASF proto-oncogene. J Cell Biol 2010; 191:87-99; PMID:20876280; http://dx.doi.org/10.1083/jcb.201001073
14. Chawla G, Lin CH, Han A, Shiue L, Ares M, Jr., Black DL. Sam68 regulates a set of alternatively spliced exons during neurogenesis. Mol Cell Biol 2009; 29:201-13; PMID:18936165; http://dx.doi.org/10.1128/MCB.01349-08
15. Paronetto MP, Messina V, Bianchi E, Barchi M, Vogel G, Moretti C, et al. Sam68 regulates translation of target mRNAs in male germ cells, necessary for mouse spermatogenesis. J Cell Biol 2009; 185:235-49; PMID:19380878; http://dx.doi.org/10.1083/jcb.200811138
16. Richard S, Torabi N, Franco GV, Tremblay GA, Chen T, Vogel G, et al. Ablation of the Sam68 RNA binding protein protects mice from age-related bone loss. PLoS Genet 2005; 1:e74; PMID:16362077; http://dx.doi.org/10.1371/journal.pgen.0010074
17. Bianchi E, Barbagallo F, Valeri C, Geremia R, Salustri A, De Felici M, et al. Ablation of the Sam68 gene impairs female fertility and gonadotropin-dependent follicle development. Hum Mol Genet 2010; 19:4886-94; PMID:20881015; http://dx.doi.org/10.1093/hmg/ddq422

18. Richard S, Vogel G, Huot ME, Guo T, Muller WJ, Lukong KE. Sam68 haploinsufficiency delays onset of mammary tumorigenesis and metastasis. Oncogene 2008; 27:548-56; PMID:17621265; http://dx.doi.org/10.1038/sj.onc.1210652
19. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev 2004; 18:1926-45; PMID:15314020; http://dx.doi.org/10.1101/gad.1212704
20. Murakami M, Ichisaka T, Maeda M, Oshiro N, Hara K, Edenhofer F, et al. mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. Mol Cell Biol 2004; 24:6710-8; PMID:15254238; http://dx.doi.org/10.1128/MCB.24.15.6710-6718.2004
21. Yeh WC, Bierer BE, McKnight SL. Rapamycin inhibits clonal expansion and adipogenic differentiation of 3T3-L1 cells. Proc Natl Acad Sci U S A 1995; 92:11086-90; PMID:7479942; http://dx.doi.org/10.1073/pnas.92.24.11086
22. Zhang HH, Huang J, Düvel K, Boback B, Wu S, Squillace RM, et al. Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. PLoS One 2009; 4:e6189; PMID:19593385; http://dx.doi.org/10.1371/journal.pone.0006189
23. Houde VP, Brûlé S, Festuccia WT, Blanchard PG, Bellmann K, Deshaies Y, et al. Chronic rapamycin treatment causes glucose intolerance and hyperlipidemia by upregulating hepatic gluconeogenesis and impairing lipid deposition in adipose tissue. Diabetes 2010; 59:1338-48; PMID:20299475; http://dx.doi.org/10.2337/db09-1324
24. Chang GR, Chiu YS, Wu YY, Chen WY, Liao JW, Chao TH, et al. Rapamycin protects against high fat diet-induced obesity in C57BL/6J mice. J Pharmacol Sci 2009; 109:496-503; PMID:19372632; http://dx.doi.org/10.1254/jphs.08215FP
25. Carnevalli LS, Masuda K, Frigerio F, Le Bacquer O, Um SH, Gandin V, et al. S6K1 plays a critical role in early adipocyte differentiation. Dev Cell 2010; 18:763-74; PMID:20493810; http://dx.doi.org/10.1016/j.devcel.2010.02.018
26. Polak P, Cybulski N, Feige JN, Auwerx J, Rüegg MA, Hall MN. Adipose-specific knockout of raptor results in lean mice with enhanced mitochondrial respiration. Cell Metab 2008; 8:399-410; PMID:19046571; http://dx.doi.org/10.1016/j.cmet.2008.09.003
27. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, et al. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature 2004; 431:200-5; PMID:15306821; http://dx.doi.org/10.1038/nature02866
28. Andrade MA, Petosa C, O'Donoghue SI, Müller CW, Bork P. Comparison of ARM and HEAT protein repeats. J Mol Biol 2001; 309:1-18; PMID:11491282; http://dx.doi.org/10.1006/jmbi.2001.4624