(BAT), the major adipose compartment during this developmental stage. Zfp423 null mice displayed a reduced mass of BAT and an increase in connective tissue intrusions. Moreover, developing white adipocyte precursor cells in the subcutaneous region were strongly reduced in Zfp423 null mice. In accordance with the finding that Zfp423 was not expressed in primary or C2C12 myoblasts, there was no obvious defect in other mesodermal lineages, as muscle and connective tissues appeared normal in these embryos. Tissue-specific or cell lineage-specific Zfp423 ablation will be required to fully understand the physiologic role of Zfp423.

Taken together, the findings presented by Gupta and colleagues bridge an existing gap in our understanding of adipocyte differentiation, which has been largely focused on the transition from preadipocytes to adipocytes using established cell lines such as the 3T3-L1 model. In this context, the new work implicates the zinc finger protein Zfp423 as a key factor to explain the critical question of how precursor cells become fated for fat (Figure 1).

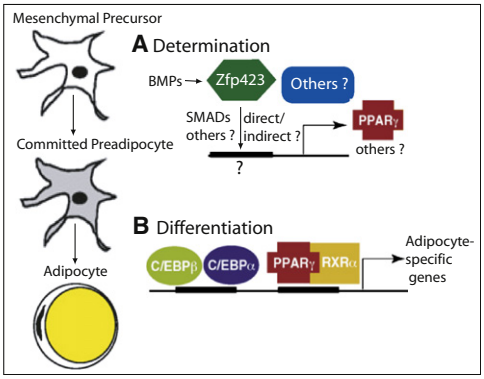

Figure 1. A Role for Zfp423 in Preadipocyte Determination

(A) Zfp423 induces expression of the master regulator of adipogenesis, PPARγ, and commits cells to become preadipocytes.

(B) PPARγ acts in a feed forward loop with C/EBP factors to maintain high expression of each other and to terminally differentiate the cells into adipocytes.

REFERENCES

Akerblad, P., Lind, U., Liberg, D., Bamberg, K., and Sigvardsson, M. (2002). Mol. Cell. Biol. *22*, 8015–8025.

Chawla, A., Schwarz, E.J., Dimaculangan, D.D., and Lazar, M.A. (1994). Endocrinology *135*, 798–800.

Cheng, L.E., Zhang, J., and Reed, R.R. (2007). Dev. Biol. *307*, 43–52.

Green, H., and Meuth, M. (1974). Cell *3*, 127–133.

Gupta, R.K., Arany, Z., Seale, P., Mepani, R.J., Ye, L., Conroe, H.M., Roby, Y.A., Kulaga, H., Reed, R.R., and Spiegelman, B.M. (2010). Nature, in press. Published online March 3, 2010. 10.1038/nature08816.

Hata, A., Seoane, J., Lagna, G., Montalvo, E., Hemmati-Brivanlou, A., and Massagué, J. (2000). Cell *100*, 229–240.

Hesslein, D.G., Fretz, J.A., Xi, Y., Nelson, T., Zhou, S., Lorenzo, J.A., Schatz, D.G., and Horowitz, M.C. (2009). Bone *44*, 537–546.

Lefterova, M.I., and Lazar, M.A. (2009). Trends Endocrinol. Metab. *20*, 107–114.

Seale, P., Kajimura, S., Yang, W., Chin, S., Rohas, L.M., Uldry, M., Tavernier, G., Langin, D., and Spiegelman, B.M. (2007). Cell Metab. *6*, 38–54.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H.M., Erdjument-Bromage, H., et al. (2008). Nature *454*, 961–967.

Tontonoz, P., Hu, E., and Spiegelman, B.M. (1994). Cell *79*, 1147–1156.

Tsai, R.Y., and Reed, R.R. (1997). J. Neurosci. *17*, 4159–4169.

Phosphatase 2A Puts the Brakes on mTORC1 Nutrient Signaling

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Nutrients such as amino acids (aa) and glucose mediate mammalian target of rapamycin complex 1 (mTORC1) signaling to control cell growth and metabolism. Recent studies (Yan et al., 2010) identify a contributor, PP2A phosphatase subunit PR61ε, in regulating the aa-sensitive input to mTORC1.

The epidemic in obesity and associated pathologies, including insulin resistance, type 2 diabetes, cardiovascular disease, and cancer (Calle and Kaaks, 2004), has been fueled by nutrient overload driven by the abundance, palatability, and low cost of food (Finkelstein et al., 2005). The intracellular serine-threonine kinase mTOR plays a critical role in mediating these pathologies. The rapamycins (specific mTOR inhibitors) have been approved for treatment of renal cancers (Houghton, 2010), and recent studies show that they increase life span, possibly through their ability to recapitulate the effects of caloric restriction (Katewa and Kapahi, 2010). mTOR is found in two structurally and functionally distinct complexes, mTORC1 and mTORC2. Although both complexes are implicated in hormonal signaling mediated by class 1 phosphatidylinositide-3OH kinase (PI3K), only mTORC1 is positively regulated by nutrients, particularly aa and glucose. In

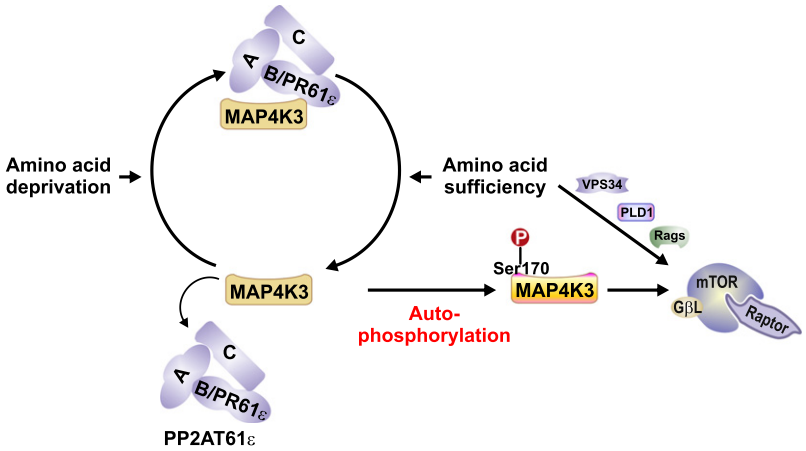

Figure 1. Regulation of MAP4K3 by PP2A
Model for the role of $PP2A_{T61\varepsilon}$ and MAP4K3 in nutrient signaling to mTORC1 (see text for details).

the absence of nutrients, mitogens and hormones fail to activate mTORC1, but not mTORC2. Thus, nutrients are essential agonists in mediating mTORC1 activation (Avruch et al., 2009). Moreover, components of the canonical PI3K signaling pathway are not utilized in mTORC1 activation by nutrients (Nobukuni et al., 2005). Instead, a distinct, dominant nutrient signaling pathway converges with the hormonal-PI3K signaling pathway to drive growth at the level of mTORC1 (Um et al., 2006).

Studies from different laboratories have identified roles for the class 3 PI3K (hVPS34), Ras-related guanosine triphosphatases (Rag GTPases), Ras homolog enriched in brain (Rheb) GTPase, and phospholipase D1 (PLD1) in nutrient signaling to mTORC1 (Avruch et al., 2009). In a previous report, Lamb and colleagues identified MAP4K3, a *Ste20*-related MAP4K, as a critical mediator of aa-induced mTORC1 activation (Findlay et al., 2007). Importantly, they demonstrated that MAP4K3 activity is regulated by aa availability but unaffected by insulin or rapamycin, placing MAP4K3 upstream, on the nutrient branch of the mTORC1 signaling pathway (Figure 1). This initial report raised several questions, including the mechanism by which aa mediate MAP4K3 activation.

In work recently published in *Molecular Cell*, Lamb and colleagues identify $PP2A_{T61\varepsilon}$ as a critical mediator of nutrient signaling to mTORC1 via MAP4K3 (Yan et al., 2010). Since many protein kinases are themselves regulated by phosphorylation, Yan et al. have now asked whether this is the case for MAP4K3 (Yan et al., 2010). To this end, they performed mass spectrometry phosphopeptide analysis on purified, ectopically expressed MAP4K3. Two phosphopeptides were identified—one included phosphorylated Ser170, from the MAP4K3 kinase domain. Subsequent mutational analysis showed this site to be critical for kinase activity. The authors then generated an antibody specific for phospho-Ser170, which did not detect endogenous MAP4K3, but did recognize inducible, ectopically expressed MAP4K3. Removal of aa from the medium for 60 min eliminated Ser170 phosphorylation, a response that was reversed within 15 min of aa readdition. Importantly, Ser170 phosphorylation is relatively unaffected by insulin treatment. Coexpression of kinase-dead and wild-type MAP4K3 as well as in vitro studies indicated that Ser170 phosphorylation is regulated by *trans*-autophosphorylation, although such studies have not ruled out a *cis*-autophosphorylation mechanism.

A further kinetic analysis revealed an acute drop in Ser170 phosphorylation within 5 min of aa depletion, suggesting the potential involvement of a phosphatase. Treatment with the phosphatase inhibitor okadaic acid at concentrations thought to be selective for PP2A blocked MAP4K3 Ser170 dephosphorylation following aa withdrawal. Consistent with this finding, addition of PP2A to purified MAP4K3 in vitro also led to Ser170 dephosphorylation. PP2A is a serine-threonine phosphatase multiprotein complex consisting of a structural subunit (PP2A-A), a regulatory or targeting subunit (PP2A-B), and a catalytic subunit (PP2A-C). PP2A-B subunits are thought to be critical for specific substrate recognition (Eichhorn et al., 2009). Mass spectrometry analysis of exogenously expressed MAP4K3 revealed that it bound to PR61ε, a regulatory/targeting subunit of PP2A. Immunoprecipitation and western blotting analyses confirmed this association. The authors then used both gain-of-function and loss-of-function approaches to investigate the role of PR61ε in aa signaling. Ectopic expression of PR61ε in the presence of aa led to inhibition of MAP4K Ser170 phosphorylation and inactivation of mTORC1, as measured by S6K1 phosphorylation. In parallel, shRNA depletion of PR61ε protein levels protected MAP4K Ser170 phosphorylation under conditions of aa deprivation. Consistent with these findings, depletion of PR61ε also protected mTORC1 signaling, as measured by S6 240/244 phosphorylation in the absence of aa. Finally, depletion of PR61ε resulted in an increase in cell size, a phenotype tightly linked to increased mTORC1 signaling.

The authors initially hypothesized that aa decrease Ser170 phosphorylation by activating PP2A via the PR61ε. However, in vitro PP2A phosphatase activity is the same regardless of whether it is assayed from cells deprived of, or stimulated by, aa. The authors then determined whether binding of PR61ε to MAP4K3 was regulated by aa by employing pull-downs with two different baits: microcystin agarose, which specifically binds to the PP2A catalytic subunit, or PR61ε. In both analyses, they found that aa deprivation induced an increase in the association of PP2A with MAP4K3. Moreover, an amino-truncated MAP4K3 (aa 1–431), which binds less well to PP2A, retained activity following aa depletion. Thus, the results support a model in which binding of PP2A to MAP4K3 is essential for the regulation of MAP4K3 by aa (Figure 1).

This study has shown that $PP2A_{T61\varepsilon}$ (PR61ε containing PP2A complex) is critical for MAP4K3 and mTORC1 regulation by aa (Figure 1). However, some questions about the $PP2A_{T61\varepsilon}$ and mTORC1 regulation still remain. The authors have shown that S6 phosphorylation is protected from aa depletion in the absence of PR61ε. However, it is yet to be

determined whether phosphorylation of S6K1 and 4E-BP1, direct substrates of mTORC1, are protected as well. This is an important issue, as PP2A is known to directly interact with S6K1 (Peterson et al., 1999). In addition, looking ahead, it would be of interest to address whether other nutrients, particularly glucose, also mediate MAP4K3 activity via $PP2A_{T61\varepsilon}$.

What is the mechanism by which increased MAP4K3 mediates mTORC1 activation? Recent studies have shown that Rag GTPases are critical components in mediating aa-induced mTORC1 activation (Avruch et al., 2009). In the absence of Rag GTPases, ectopic expression of MAP4K3 failed to increase mTORC1 activation, prompting the authors to suggest that Rag GTPases act downstream of MAP4K3. However, this does not exclude the possibility of a parallel pathway or even that Rag GTPases regulate MAP4K3 activity, as in the absence of Rag GTPases, ectopically expressed MAP4K3 may be inactive. The authors do not detect binding of Rag GTPases to MAP4K3, nor do the Rag GTPases serve as an in vitro MAP4K3 substrate. Analyses of Rag GTP loading and the ability of Rags to bind to mTORC1 upon ectopic expression or depletion of MAP4K3 would yield a more definitive picture of the relationship between MAP4K3 and the Rag GTPases. In addition, a deeper understanding of the interplay between MAP4K3, Rags, hVPS34, and PLD1 will be critical in elucidating the underlying mechanisms by which nutrients control mTORC1 signaling.

Recent studies suggest that PP2A acts as a tumor suppressor (Eichhorn et al., 2009). Moreover, hyperactivation of mTORC1 signaling activates a negative feedback loop from S6K1, which acts to suppress insulin signaling, leading to insulin resistance (Um et al., 2006). The mTORC1 signaling pathway may be a critical target of PP2A in suppressing both tumorigenesis and insulin resistance. Identifying the direct target of MAP4K3 in mediating mTORC1 activation and understanding the mechanisms by which aa affect the association of PR61ε and MAP4K3 would aid in assessing the potential of these components as therapeutic targets for the treatment of cancer and type 2 diabetes.

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REFERENCES

Avruch, J., Long, X., Ortiz-Vega, S., Rapley, J., Papageorgiou, A., and Dai, N. (2009). Am. J. Physiol. Endocrinol. Metab. *296*, E592–E602.

Calle, E.E., and Kaaks, R. (2004). Nat. Rev. Cancer *4*, 579–591.

Eichhorn, P.J., Creyghton, M.P., and Bernards, R. (2009). Biochim. Biophys. Acta *1795*, 1–15.

Findlay, G.M., Yan, L., Procter, J., Mieulet, V., and Lamb, R.F. (2007). Biochem. J. *403*, 13–20.

Finkelstein, E.A., Ruhm, C.J., and Kosa, K.M. (2005). Annu. Rev. Public Health *26*, 239–257.

Houghton, P.J. (2010). Clin. Cancer Res. *16*, 1368–1372.

Katewa, S.D., and Kapahi, P. (2010). Aging Cell *9*, 105–112.

Nobukuni, T., Joaquin, M., Roccio, M., Dann, S.G., Kim, S.Y., Gulati, P., Byfield, M.P., Backer, J.M., Natt, F., Bos, J.L., et al. (2005). Proc. Natl. Acad. Sci. USA *102*, 14238–14243.

Peterson, R.T., Desai, B.N., Hardwick, J.S., and Schreiber, S.L. (1999). Proc. Natl. Acad. Sci. USA *96*, 4438–4442.

Um, S.H., D'Alessio, D., and Thomas, G. (2006). Cell Metab. *3*, 393–402.

Yan, L., Mieulet, V., Burgess, D., Findlay, G.M., Sully, K., Procter, J., Goris, J., Janssens, V., Morrice, N.A., and Lamb, R.F. (2010). Mol. Cell *37*, 633–642.