Putting the brakes on FOXO1 in fat

Jane J Kim^{1,2}, Wuqiang Fan³ and Jerrold M Olefsky^{3,*}¹Department of Pediatrics, University of California at San Diego, La Jolla, CA, USA, ²Rady Children's Hospital of San Diego, CA, USA and ³Department of Medicine, University of California at San Diego, La Jolla, CA, USA

*Correspondence to: jolefsky@ucsd.edu

The EMBO Journal (2012) 31, 2240–2241. doi:10.1038/emboj.2012.116; Published online 17 April 2012

The Foxo1 forkhead transcription factor is a critical negative regulator of insulin action. In this issue of The EMBO Journal, Nakae and colleagues (Nakae *et al*, 2012) describe a novel Foxo1 Co-Repressor (FCoR) protein in adipocytes, showing that FCoR enhances the acetylation of Foxo1 to inhibit Foxo1 activity, promote adipocyte differentiation, and improve insulin sensitivity.

The FoxO forkhead transcription factors have emerged as important targets of insulin and growth factor action, participating in a diverse array of biological processes. The Foxo1 isoform is a key regulator of insulin action, integrating cellular responses to changes in nutrient availability and oxidative stress (Accili and Arden, 2004). In the liver, Foxo1 induces gluconeogenesis, impairs mitochondrial metabolism, and promotes lipogenesis. In skeletal muscle, Foxo1 controls the shift from glucose to lipids as a source of fuel during fasting (Furuyama *et al*, 2003). In adipose tissue, Foxo1 inhibits the differentiation of adipocytes and augments insulin resistance via trans-repression of PPAR γ (Dowell *et al*, 2003; Nakae *et al*, 2003; Fan *et al*, 2009; Kim *et al*, 2009). Overall, Foxo1 deletion leads to improved insulin sensitivity (Kitamura *et al*, 2001).

Foxo1 is controlled by post-translational modifications, such as phosphorylation and acetylation, which affect its subcellular location, DNA-binding properties, and transcriptional activity. Following its phosphorylation and activation by insulin and other growth factors, Akt can migrate to the nucleus where it directly phosphorylates Foxo1 (Biggs *et al*, 1999). Once phosphorylated, Foxo1 is excluded from the nucleus, effectively silencing its transcriptional activity.

Foxo1 acetylation provides a second layer of regulation. Foxo1 can be acetylated by CBP and P/CAF and deacetylated by class I–III histone deacetylases such as Sirt1 and Sirt2. Acetylation inhibits Foxo1 activity by three distinct mechanisms: (1) acetylation of lysine residues within the forkhead domain impairs its ability to bind DNA; (2) acetylation of Foxo1 increases Akt-mediated phosphorylation of Ser-253 with subsequent nuclear exclusion; and (3) acetylation of Foxo1 causes nuclear exclusion independent of its phosphorylation state (Matsuzaki *et al*, 2005; Qiang *et al*, 2010). In contrast, Foxo1 deacetylation promotes nuclear retention.

In an experimental tour-de-force, Nakae *et al* (2012), further illuminate how Foxo1 function is regulated in adipose tissue through the discovery of a novel Foxo1 co-repressor protein (Figure 1). Using yeast two-hybrid

screening of a mouse adipocyte cDNA library, they identified a novel adipose-specific Foxo1 binding partner, named 'FCoR' (Foxo1 Co-Repressor). They show that FCoR is a 106-amino acid protein expressed in both white (WAT) and brown (BAT) adipose tissue, and binds to endogenous Foxo1. The authors also demonstrate that FCoR expression is required for adipocyte differentiation, presumably by repressing Foxo1.

Figure 1 In the basal state, Foxo1 is localized to the nucleus where it is transcriptionally active. During feeding, insulin signalling results in Akt-mediated phosphorylation and export of nuclear Foxo1 to the cytosol. In the cytosol, Foxo1 can bind to FCoR. During fasting, activated PKA directly phosphorylates FCoR, which then undergoes translocation to the nucleus. In the nucleus, FCoR directly acetylates Foxo1 through its intrinsic acetyltransferase activity. FCoR also disrupts the protein–protein interaction between Sirt1 and Foxo1, reducing deacetylation. Acetylation impairs the DNA-binding ability of Foxo1 and enhances its nuclear export, thereby repressing its transcriptional activity.

Through a series of reporter assays, the authors show that FCoR inhibits Foxo1 transactivation and that both FCoR and Foxo1 co-localize in the nucleus. The subcellular localization of FCoR is regulated by PKA, which phosphorylates cytosolic FCoR at Thr-93, resulting in its nuclear import. Importantly, the authors elucidated the mechanism by which FCoR represses Foxo1 activity by demonstrating that FCoR enhances acetylation of Foxo1 by two mechanisms. First, FCoR directly acetylates Foxo1 through its intrinsic acetyltransferase activity. Second, FCoR disrupts the protein–protein interaction between Foxo1 and Sirt1, thereby reducing deacetylation.

To investigate the function of FCoR *in vivo*, the authors generated adipocyte-specific transgenic mice and found that FCoR overexpression in WAT improved glucose tolerance and insulin sensitivity. As predicted, the acetylation of endogenous Foxo1 was increased in WAT in association with decreased expression of several Foxo1 target genes. Surprisingly, the FCoR transgenic mice were lean compared with WT controls, even though their experiments indicate that FCoR is necessary for adipocyte differentiation. This indicates that the lean phenotype could be secondary to effects of FCoR on targets other than Foxo1.

Loss of function studies in FCoR KO mice further revealed its role in WAT. FCoR KO mice were glucose intolerant and insulin resistant, despite being leaner than WT controls. Interestingly, inflammatory gene expression was increased in WAT, which could account for the development of insulin resistance. Although not specifically addressed in this paper, the increased inflammation likely results from enhanced Foxo1 activation of inflammatory signalling pathways within WAT, as Foxo1 can serve as a proinflammatory transcription factor (Fan *et al*, 2010).

Overall Nakae *et al* (2012), identify FCoR as an important new metabolic regulator in adipocytes through its direct

repression of Foxo1. FCoR binds to Foxo1 in the nucleus, inhibiting Foxo1 transcriptional activity by enhancing acetylation. As phosphorylation of Foxo1 controls its subcellular location, it will be important in future studies to determine whether FCoR can modulate Akt-mediated Foxo1 phosphorylation.

This study raises many intriguing questions regarding the physiological role of FCoR and how cellular responses are coordinated in response to fasting or fed states. For instance, fasting activates PKA phosphorylation of FCoR and its translocation to the nucleus. Since Foxo1 is transcriptionally active in the nucleus when insulin levels are low, does FCoR serve as a brake to prevent excessive Foxo1 activity during nutrient restriction? In contrast, feeding results in insulin/Akt-mediated nuclear export of Foxo1. The authors also show that FCoR can bind to Foxo1 in the cytosol. What function, if any, does the interaction between FCoR and Foxo1 serve in the cytosol?

Notably, Foxo1 is known to repress ligand-dependent transcriptional activation of PPAR γ in adipocytes (Dowell *et al*, 2003; Fan *et al*, 2009). However, the authors observed that FCoR had no effect on PPAR γ transcriptional effects in a reporter assay. Despite this, FCoR knockdown suppressed and FCoR overexpression increased PPAR γ expression in WAT. Future efforts to identify FCoR-binding proteins other than Foxo1 may further illuminate how FCoR regulates PPAR γ . No matter what any of these future studies reveal, it is clear that this new work from Nakae *et al* (2012) presents a significant step forward in our understanding of Foxo1 regulation and provides a potential new target in the treatment of insulin resistance.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Nakae J, Cao Y, Hakuno F, Takemori H, Kawano Y, Sekiko R, Abe T, Kiyonari H, Tanaka T, Sakai J, Takahashi S-I, Itoh H (2012) Novel repressor regulates insulin sensitivity through interaction with Foxo1. *EMBO J* **31**: 2275–2295
- Accili D, Arden KC (2004) FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell* **117**: 421–426
- Furuyama T, Kitayama K, Yamashita H, Mori N (2003) Forkhead transcription factor FOXO1 (FKHR)-dependent induction of PDK4 gene expression in skeletal muscle during energy deprivation. *Biochem J* **375**: 365–371
- Fan W, Imamura T, Sonoda N, Sears DD, Patsouris D, Kim JJ, Olefsky JM (2009) FOXO1 transrepresses PPAR γ transactivation, coordinating an insulin-induced feed-forward response in adipocytes. *J Biol Chem*
- Kim JJ, Li P, Huntley J, Chang JP, Arden KC, Olefsky JM (2009) FoxO1 haploinsufficiency protects against high-fat diet-induced insulin resistance with enhanced peroxisome proliferator-activated receptor gamma activation in adipose tissue. *Diabetes* **58**: 1275–1282
- Nakae J, Kitamura T, Kitamura Y, Biggs 3rd WH, Arden KC, Accili D (2003) The forkhead transcription factor Foxo1 regulates adipocyte differentiation. *Dev Cell* **4**: 119–129
- Dowell P, Otto TC, Adi S, Lane MD (2003) Convergence of peroxisome proliferator-activated receptor gamma and Foxo1 signaling pathways. *J Biol Chem* **278**: 45485–45491
- Kitamura T, Kido Y, Nef S, Merenmies J, Parada LF, Accili D (2001) Preserved pancreatic beta-cell development and function in mice lacking the insulin receptor-related receptor. *Mol Cell Biol* **21**: 5624–5630
- Biggs 3rd WH, Meisenhelder J, Hunter T, Cavenne WK., Arden KC (1999) Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. *Proc Natl Acad Sci USA* **96**: 7421–7426
- Matsuzaki H, Daitoku H, Hatta M, Aoyama H, Yoshimochi K, Fukamizu A (2005) Acetylation of Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. *Proc Natl Acad Sci USA* **102**: 11278–11283
- Qiang L, Banks AS, Accili D (2010) Uncoupling of acetylation from phosphorylation regulates FoxO1 function independent of its subcellular localization. *J Biol Chem* **285**: 27396–27401
- Fan W, Morinaga H, Kim JJ, Bae E, Spann NJ, Heinz S, Glass CK, Olefsky JM (2010) FoxO1 regulates Tlr4 inflammatory pathway signalling in macrophages. *EMBO J* **29**: 4223–4236