The activity of transcription factor FoxO1 is regulated by phosphorylation-dependent nuclear exclusion and deacetylation-dependent nuclear retention. It is unclear whether and how these two post-translational modifications affect each other. To answer this question, we expressed FoxO1 cDNAs with combined mutations of phosphorylation and acetylation sites in HEK-293 cells and analyzed their subcellular localization patterns. We show that mutations mimicking the deacetylated state (KQ series) render FoxO1 more sensitive to Akt-mediated phosphorylation and nuclear exclusion and can reverse the constitutively nuclear localization of phosphorylation-defective FoxO1. Conversely, mutations mimicking the deacetylated state (KR series) promote FoxO1 nuclear retention. Oxidative stress and the Sirt1 activator resveratrol are thought to promote FoxO1 deacetylation and nuclear retention, thus increasing its activity. Accordingly, FoxO1 deacetylation was required for the effect of oxidative stress (induced by H$_2$O$_2$) to retain FoxO1 in the nucleus. H$_2$O$_2$ also inhibited FoxO1 phosphorylation on Ser-253 and Thr-24, the key insulin-regulated sites, irrespective of its acetylation. In contrast, the effect of resveratrol was independent of FoxO1 acetylation and its phosphorylation on Ser-253 and Thr-24, suggesting that resveratrol acts on FoxO1 in a Sirt1- and Akt-independent manner. The dissociation of deacetylation from dephosphorylation in H$_2$O$_2$-treated cells indicates that the two modifications can occur independently of each other. It can be envisaged that FoxO1 exists in multiple nuclear forms with distinct activities depending on the balance of deacetylation and phosphorylation.

FoxO1 and its closely related isoforms FoxO3A and FoxO4 are transcription factors characterized by a conserved winged helix (“forkhead”) DNA binding domain. Genetic epistasis experiments in Caenorhabditis elegans demonstrated a role for these proteins in insulin receptor signaling, spawning studies of their contribution to mammalian metabolism, cellular differentiation, and transformation (1). It is now recognized that FoxOs are critical regulators of hepatic gluconeogenesis (2, 3) and pancreatic β-cell function (4–8), in addition to differentiation of myotubes (9–11) and adipocytes (12). Moreover, the C. elegans FoxO ortholog DAF-16 is required for life extension caused by DAF-2 (insulin receptor) mutations, suggesting that FoxO has a role in longevity (13, 14).

FoxO activity is regulated by post-translational modifications that affect primarily its subcellular localization (15). Insulin and growth factor signaling inhibit FoxO via Akt-dependent phosphorylation and nuclear exclusion (16–18). Several additional serine/threonine kinases, such as Mst1 (19), Ink (20), and Sgk promote or inhibit FoxO via nuclear translocation (20–22) or nuclear exclusion, respectively (23–25). A second regulatory layer is FoxO acetylation by p300, CBP (cAMP-response element-binding protein–binding protein), and PCAF (p300/CBP-associated factors) in response to oxidative stress or DNA binding (26–28), followed by deacetylation by class I and II histone deacetylases (26, 28–30), including Sirt1, the NAD$^+$-dependent deacetylase encoded by the ortholog of yeast longevity gene Sir2 (31).

The effects of phosphorylation and acetylation on FoxO function have been studied extensively but separately. However, these two modifications are likely to occur concurrently in vivo and to reciprocally affect each other. In this study, we generated an allelic series of FoxO1 mutants containing changes to both acetylation and phosphorylation sites and analyzed their regulation in response to physiologic (insulin) and pathophysiologic cues (oxidative stress, resveratrol) to explore the reciprocal regulation of acetylation and phosphorylation and their combined effects on FoxO1 cellular localization and biological functions.

**EXPERIMENTAL PROCEDURES**

*Materials—Dulbecco’s modified Eagle’s medium with 4.5 g/liter glucose, fetal bovine serum, calf serum, trypsin/EDTA, and phosphate-buffered saline were purchased from Mediatech (Manassas, VA). Insulin, H$_2$O$_2$, resveratrol, nicotinamide, and cycloheximide were purchased from Sigma. Akt-1/2 from EMD, microcystin-LR from Cayman, and leptomycin B from LC Laboratories. Anti-FLAG (M2) affinity gel was purchased from Sigma. Anti-phospho-S253, phospho-T24 FoxO1, and phospho-Akt antibodies (T308) were purchased from Cell Signaling. Anti-GFP, anti-tubulin, and anti-FLAG antibodies were purchased from Santa Cruz Biotechnology.*

*Plasmids, Adenoviruses, and Cell Culture—cDNAs encoding murine FoxO1 and carrying the following mutations, T24A, T24A-KQ, T24A-KR, and S253A, were subcloned into pEGFP-N1 to generate FoxO1-GFP fusion proteins. Plasmids*
Effects of Acetylation Site Mutations on FoxO1 Subcellular Localization—We studied the effect of mutating acetylation sites on insulin-induced FoxO1 subcellular translocation. To this end, we transfected wild type (WT), KQ, or KR FoxO1-GFP fusion proteins into HEK-293 cells. WT localized to the nucleus in serum-free medium and translocated to the cytoplasm upon insulin stimulation. The Akt inhibitor Akti-1/2 inhibited this process (33) (Fig. 1B). Conversely, the KQ mutant was predominantly cytoplasmic, regardless of whether cells were incubated in serum-free medium or in the presence of insulin and Akt inhibitor. The KR mutant translocated to the cytoplasm after insulin stimulation in a WT-like fashion and was retained in the nucleus after Akti-1/2 treatment, whereas the S253A mutant was constitutively nuclear under all conditions tested (Fig. 1B) (24).

We next examined whether acetylation trumps phosphorylation as a signal for FoxO1 retention in the nucleus. To this end, we compared the phosphorylation-defective mutant ADA (12) with combined phosphorylation/acetylation site mutants, ADA-KQ or ADA-KR. ADA was constitutively nuclear regardless of the culture conditions (Fig. 2A). Surprisingly, ADA-KQ had predominantly cytoplasmic localization, whereas ADA-KR was retained in the nucleus (Fig. 2A). These data indicate that acetylation trumps phosphorylation as a signal regulating FoxO1 cellular localization. Inhibition of nuclear export by leptomycin B resulted in nuclear accumulation of both KQ and ADA-KQ, indicating that acetylation doesn’t prevent nuclear targeting of FoxO1 but likely accelerates its export to the cytoplasm or retards its nuclear import (Fig. 2B).

To rule out that mutation of the phosphorylation sites affected FoxO1 acetylation, we measured acetyl-FoxO1 levels in the phosphorylation site mutants, S253A and T24A. As the former is unaffected by insulin treatment, we measured only basal acetylation in the absence of insulin; in the latter, we compared acetylation levels in the absence and presence of insulin. However in neither case did we observe changes to FoxO1 acetylation (Fig. 2C).

RESULTS AND DISCUSSION

Generation of FoxO1 Mutants—In addition to the previously described constitutively acetylated (FoxO1-KQ, in which lysine at amino acid residues 219, 242, 245, 259, 262, 271, and 291 is replaced with glutamine) and constitutively decetylated mutants (FoxO1-KR, in which the same lysine residues are replaced with arginine) (Fig. 1A) (8), we generated an allelic series in which the KR and KQ mutations were introduced along with the following mutations of the three main phosphorylation sites: T24A, S253A, or combined T24A/S253D/S316A (ADA mutant) (12). Ser-253 is the main Akt site, whereas Thr-24 is phosphorylated by an insulin-activated kinase(s) distinct from Akt (23, 25). We generated the mutants KR, KQ, S253A, T24A-KQ, T24A-KR, ADA-KQ, and ADA-KR as GFP fusion proteins to facilitate their detection by fluorescence microscopy (30). For simplicity, we omit “FoxO1-GFP” from the nomenclature.

Effects of Acetylation Site Mutations on Insulin-induced FoxO1 Phosphorylation—To understand why acetylation promotes FoxO1 nuclear exclusion, we examined whether it affects phosphorylation of Ser-253, the site required for insulin-dependent nuclear translocation (24). To avoid the potential confounding effects of Thr-24 phosphorylation on subcellular localization (24), we measured Ser-253 phosphorylation in T24A-KQ and T24A-KR mutants following exposure of cells to different doses of insulin. Insulin-induced Ser-253 phosphorylation of the T24A mutant paralleled Akt phosphorylation in a dose-dependent manner, with an $ED_{50} \sim 0.3 \text{ nM}$ (Fig. 3). In contrast, the $ED_{50}$ for Ser-253 phosphorylation decreased to <0.15 nM in the T24A-KQ mutant and rose to >1.5 nM in the T24A-KR mutant, resulting in a 10-fold difference between the two mutants. Interestingly, levels of the T24A-KQ mutant decreased in insulin-treated cells. Based on prior studies, this is
likely to reflect increased protein degradation through the proteasome (8). This process was reversed by Akt inhibition, as was Ser-253 phosphorylation (Fig. 3). These data indicate that acetylation increases FoxO1 sensitivity to Akt phosphorylation and degradation, suggesting that FoxO1 nuclear exclusion and protein turnover are integrated through acetylation-based mechanisms.

Uncoupling of Acetylation from Phosphorylation following H$_2$O$_2$-induced Oxidative Stress—Oxidative stress and the polyphenol resveratrol promote FoxO1 nuclear retention (30). Their effects have been ascribed to FoxO1 deacetylation (27, 28, 34). However, the data in Fig. 3 raise the possibility that they also inhibit FoxO1 phosphorylation or promote its dephosphorylation. To answer this question, we used acetylation site FoxO1 mutants to examine FoxO1 localization and phosphorylation following incubation of cells with insulin and H$_2$O$_2$, a chemical agent used to mimic oxidative stress (35), or insulin and resveratrol, a Sirt1 and AMP-activated protein kinase agonist (29, 30). Addition of H$_2$O$_2$ to insulin-treated cells prevented FoxO1 nuclear export. This effect was reversed by the constitutively acetylated KQ mutant but not by the deacetylated KR mutant, indicating that H$_2$O$_2$ promotes FoxO1 deacetylation or requires that FoxO1 be deacetylated to keep it in the nucleus (Fig. 4A). Resveratrol also prevented FoxO1 nuclear exclusion in response to insulin but, unlike H$_2$O$_2$, failed to prevent nuclear exclusion of either KQ or KR mutants (Fig. 4A), indicating that its effects are independent of FoxO1 acetylation.

Next we compared the effects of H$_2$O$_2$ and resveratrol on insulin-dependent phosphorylation of Ser-253 and Thr-24. Insulin promoted FoxO1 phosphorylation on both sites. Addition of H$_2$O$_2$ to insulin decreased phosphorylation of both sites. The effect of H$_2$O$_2$ was preserved in the KQ and KR mutants, indicating that it is independent of FoxO1 acetylation (Fig. 4B). Addition of resveratrol to insulin also decreased insulin-dependent phosphorylation of Ser-253 and Thr-24. Insulin promoted FoxO1 phosphorylation on both sites. Addition of H$_2$O$_2$, to insulin decreased phosphorylation of both sites. The effect of H$_2$O$_2$ was preserved in the KQ and KR mutants, indicating that it is independent of FoxO1 acetylation (Fig. 4B). Addition of resveratrol to insulin also decreased insulin-dependent Ser-253 and Thr-24 phosphorylation in WT FoxO1, but not in the KQ and KR mutants (Fig. 4B). Neither H$_2$O$_2$ nor resveratrol affected insulin-induced Akt phosphorylation to a significant extent (Fig. 4B), and their effect on Ser-253 phosphorylation was independent of changes in FoxO1 protein levels, as indicated by the fact that they retained their ability to decrease Ser(P)-253 in the presence of the protein synthesis inhibitor, cycloheximide (Fig. 4C).

From these experiments, we concluded that the effects of resveratrol are mediated neither by changes in FoxO1 acetylation nor by dephosphorylation of Ser-253 and Thr-24. In contrast, H$_2$O$_2$ promotes FoxO1 nuclear retention through a dual
mechanism: deacetylation and reduced insulin-dependent phosphorylation of Ser-253 and Thr-24. Interestingly, the two effects can occur independently. Thus, despite their apparent similarities, resveratrol and H2O2 affect FoxO1 activity in mechanistically distinct fashions.

H2O2 might promote FoxO1 dephosphorylation either by preventing access of the relevant kinases to these sites or by easing access by the relevant phosphatases. To address this question, we examined whether the ability of H2O2 to prevent FoxO1 phosphorylation on Ser-253 was reversed by inhibition of PP2A, a FoxO1 Ser-253 phosphatase (36). H2O2 decreased Ser-253 phosphorylation, and its effect was partly reversed by the PP2A inhibitor microcystin-LR (Fig. 5A). The ability of microcystin-LR to offset H2O2 inhibition of Ser-253 phosphorylation was preserved in the KQ, but not in the KR, mutant (Fig. 5A). Given that the KR mutant is predominantly nuclear, these data are consistent with the interpretation that PP2A-dependent FoxO1 dephosphorylation occurs outside the nucleus. The fact that the effect of microcystin-LR is partial indicates that H2O2 also regulates other phosphatases or prevents access of Akt to FoxO1.

The ability of H2O2 to promote FoxO1 nuclear retention was preempted, but not reversed, by the deacetylase inhibitor nicotinamide (8). Thus, cell pretreatment with nicotinamide blocked H2O2-induced nuclear translocation, but addition of nicotinamide after H2O2 treatment was unable to reverse this effect (Fig. 5B), indicating that FoxO1 acetylation can prevent its nuclear entry but cannot promote its nuclear exclusion.

The effect of resveratrol, like that of H2O2, appears to entail reduced FoxO1 phosphorylation on Ser-253 and Thr-24. Decreased Ser-253 phosphorylation in resveratrol-treated cells was partly reversed by microcystin-LR (Fig. 5C), indicating that part of the effect of resveratrol is PP2A-dependent.

14-3-3 participates in nucleocytoplasmic shuttling of FoxO by binding phosphorylated Thr-24 and Ser-253 (37, 38) and given the tight relationship between the phosphorylation of these sites and acetylation, we asked whether acetylation was preserved in the KQ, but not in the KR, mutant (Fig. 5A). Given that the KR mutant is predominantly nuclear, these data are consistent with the interpretation that PP2A-dependent FoxO1 dephosphorylation occurs outside the nucleus. The fact that the effect of microcystin-LR is partial indicates that H2O2 also regulates other phosphatases or prevents access of Akt to FoxO1.
FoxO1 Acetylation and Phosphorylation

affected binding of 14-3-3 to the Ser-253 site. Using immuno-precipitation of FLAG-tagged T24A, T24A-KQ, and T24A-KR mutants, followed by immunoblotting with anti-14-3-3 antibody, we observed that insulin-induced phosphorylation of Ser-253 was associated with increased binding of T24A to 14-3-3 (Fig. 6). Constitutively deacetylated T24A-KR bound 14-3-3 more efficiently than T24A (Fig. 6, lanes WT and KR under + Insulin), even as its phosphorylation on Ser-253 was reduced. The constitutively acetylated T24A-KQ mutant also showed increased 14-3-3 binding, but, unlike the KR mutant, it was associated with increased Ser-253 phosphorylation (Fig. 6, compare lanes WT and KQ under − Insulin with the same lanes under + Insulin). These data indicate that FoxO1 binding to 14-3-3 is also modulated by its acetylation state, lending further support to the idea that acetylation affects FoxO1 nucleocytoplastic shuttling.

Conclusions—The goal of this study was to examine the reciprocal regulation of two primary posttranslational modifications of FoxO1, acetylation and phosphorylation, and their combined effects on FoxO1 function. Using constitutively deacetylated (KR) and acetylated (KQ) mutants, we show that acetylation causes a leftward shift in the dose-response curve for insulin-induced FoxO1 phosphorylation, whereas deacetylation causes a rightward shift. As a result, the two mutants differ by ~10-fold in their insulin sensitivity, suggesting that acetylation is a major determinant of FoxO1 activity in vivo.

A new finding of the present study is that the two modifications, acetylation and phosphorylation, can be uncoupled from each other. Using H2O2 to mimic oxidative stress and induce FoxO1 deacetylation, we show that H2O2 can antagonize insulin signaling by promoting either FoxO1 deacetylation or dephosphorylation of Ser-253 and Thr-24, the former in part through the serine/threonine phosphatase PP2A. In either case, the expectation is that FoxO1 will be retained in the nucleus.

In contrast, and somewhat surprisingly, resveratrol promotes FoxO1 nuclear localization independent of acetylation, as well as of Ser-253 and Thr-24 phosphorylation. These data support the recent observation that resveratrol acts by deacetylation-independent mechanisms (e.g. AMP-activated protein kinase activation) (39). We cannot exclude the possibility that resveratrol is unable to induce dephosphorylation of the KQ and KR mutants, because of structural alterations caused by the replacement of acetyllysine with arginine or glutamine.

The uncoupling of FoxO1 phosphorylation from acetylation in H2O2-treated cells has important ramifications for FoxO1 nuclear function. In fact, it has been shown that acetylation affects FoxO1 affinity to bind its DNA targets (8, 26) and may thus favor DNA binding-independent modalities of FoxO1 function, e.g. cell differentiation versus replication and metabolism (10). Likewise, it has previously been demonstrated that phosphorylation affects transactivation properties of FoxO1 (32), making it theoretically possible that FoxO1 be nuclear and inactive.

In conclusion, the findings that phosphorylated FoxO1 can be retained in the nucleus by decreasing its acetylation and, conversely, that dephosphorylated FoxO1 can be targeted to the cytoplasm through increased acetylation underscore that phosphorylation and acetylation are regulated through partly overlapping, and partly independent, mechanisms and suggest new research directions to develop agents that modulate pleiotropic functions of FoxOs.

Acknowledgment—We thank members of the Accili laboratory for helpful discussions and advice.

REFERENCES

1. Accili, D., and Arden, K. C. (2004) Cell 117, 421–462
2. Nakae, J., Biggs, W. H., 3rd, Kitamura, T., Cavenee, W. K., Wright, C. V., Arden, K. C., and Accili, D. (2002) Nat. Genet. 32, 245–253
3. Nakae, J., Kitamura, T., Silver, D. L., and Accili, D. (2001) J. Clin. Invest. 108, 1359–1367
4. Buteau, J., and Accili, D. (2007) Diabetes. Obes. Metab. 9, Suppl. 2, 140–146
5. Buteau, J., Shlien, A., Foisy, S., and Accili, D. (2007) J. Biol. Chem. 282, 287–293
6. Kitamura, T., Kitamura, Y. I., Kobayashi, M., Kikuchi, O., Sasaki, T., De-pinho, R. A., and Accili, D. (2009) Mol. Cell. Biol. 29, 4417–4430
7. Kitamura, T., Nakae, J., Kitamura, Y., Kido, Y., Biggs, W. H., 3rd, Wright, C. V., White, M. F., Arden, K. C., and Accili, D. (2002) J. Clin. Invest. 110, 1839–1847
8. Kitamura, Y. I., Kitamura, T., Kruse, J. P., Raum, J. C., Stein, R., Gu, W., and Accili, D. (2005) Cell Metab. 2, 153–163
9. Hribal, M. L., Nakae, J., Kitamura, T., Shutter, J. R., and Accili, D. (2003) J. Cell Biol. 162, 535–541
10. Kitamura, T., Kitamura, Y. I., Funahashi, Y., Shawyer, C. J., Castrillon, D. H., Kollipara, R., DePinho, R. A., Kitajewski, J., and Accili, D. (2007) J. Clin. Invest. 117, 2477–2485
11. Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., Walsh, K., Schiaffino, S., Lecker, S. H., and Goldberg, A. L. (2004) Cell 117, 399–412
12. Nakae, J., Kitamura, T., Kitamura, Y., Biggs, W. H., 3rd, Arden, K. C., and Accili, D. (2003) Dev. Cell 4, 119–129
13. Lin, K., Dormann, J. B., Rodan, A., and Kenyon, C. (1997) Science 278, 1319–1322
14. Ogg, S., Paradis, S., Gottlieb, S., Patterson, G. L., Lee, L., Tissenbaum, H. A., and Ruvkun, G. (1997) Nature 389, 994–999
15. Vogt, P. K., Jiang, H., and Aoki, M. (2005) Cell Cycle 4, 908–913
16. Biggs, W. H., 3rd, Meisenhelder, J., Hunter, T., Cavenee, W. K., and Arden, K. C. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 7421–7426
17. Brunet, A., Bonni, A., Zignmond, M. J., Lin, M. Z., Joo, P., Hu, L. S., Anderson, M. J., Arden, K. C., Blenis, J., and Greenberg, M. E. (1999) Cell 96, 857–868
18. Nakae, J., Park, B. C., and Accili, D. (1999) J. Biol. Chem. 274, 15982–15985
19. Lehtinen, M. K., Yuan, Z., Boag, P. R., Yang, Y., Villén, J., Becker, E. B., DiBacco, S., de la Iglesia, N., Gygi, S., Blackwell, T. K., and Bonni, A. (2006) Cell 125, 987–1001
20. Essers, M. A., Weijzen, S., de Vries-Smits, A. M., Saarloos, I., de Ruiter, N. D., Bos, J. L., and Burgering, B. M. (2004) EMBO J. 23, 4802–4812
21. Asada, S., Daitoku, H., Matsuzaki, H., Saito, T., Sudo, T., Mukai, H., Iwashita, S., Kako, K., Kishi, T., Kasuya, Y., and Fukamizu, A. (2007) Cell Signal. 19, 519–527
22. Kawamori, D., Kaneto, H., Nakatani, Y., Matsuoka, T. A., Matsuhisa, M., Hori, M., and Yamasaki, Y. (2006) J. Biol. Chem. 281, 1091–1098
23. Brunet, A., Park, J., Tran, H., Hu, L. S., Hemmings, B. A., and Greenberg, M. E. (2001) Mol. Cell. Biol. 21, 952–965
24. Nakae, J., Barr, V., and Accili, D. (2000) EMBO J. 19, 989–996
25. Nakae, J., Kitamura, T., Ogawa, W., Kasuga, M., and Accili, D. (2001) Biochemistry 40, 11768–11776
26. Daitoku, H., Hatta, M., Matsuzaki, H., Aratani, S., Ohshima, T., Miyagishi, M., Nakajima, T., and Fukamizu, A. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 10042–10047
27. Fukuoka, M., Daitoku, H., Hatta, M., Matsuzaki, H., Umemura, S., and Fukamizu, A. (2003) Int. J. Mol. Med. 12, 503–508
28. van der Horst, A., Tertoolen, L. G., de Vries-Smits, L. M., Frye, R. A., Medema, R. H., and Burgering, B. M. (2004) J. Biol. Chem. 279, 28873–28879
29. Brunet, A., Sweeney, L. B., Sturgill, J. F., Chua, K. F., Greer, P. L., Lin, Y., Tran, H., Ross, S. E., Mostoslavsky, R., Cohen, H. Y., Hu, L. S., Cheng, H. L., Jedrychowski, M. P., Gygi, S. P., Sinclair, D. A., Alt, F. W., and Greenberg, M. E. (2004) Science 303, 2011–2015
30. Frescas, D., Valenti, L., and Accili, D. (2005) J. Biol. Chem. 280, 20589–20595
31. Imai, S., Armstrong, C. M., Kaerberlein, M., and Guarente, L. (2000) Nature 403, 795–800
32. Tomizawa, M., Kumar, A., Perrot, V., Nakae, J., Accili, D., Rechler, M. M., and Kumaro, A. (2000) J. Biol. Chem. 275, 7289–7295
33. Logie, L., Ruiz-Alcaraz, A. J., Keane, M., Woods, Y. L., Bain, J., Marquez, R., Alessi, D. R., and Sutherland, C. (2007) Diabetes 56, 2218–2227
34. Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Liner, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P., and Auwerx, J. (2006) Cell 127, 1109–1122
35. Nemoto, S., and Finkel, T. (2002) Science 295, 2450–2452
36. Yan, L., Lavin, V. A., Moser, L. R., Cui, Q., Kanies, C., and Yang, E. (2008) J. Biol. Chem. 283, 7411–7420
37. Obsil, T., Ghirlando, R., Anderson, D. E., Hickman, A. B., and Dyda, F. (2003) Biochemistry 42, 15264–15272
38. Rena, G., Prescott, A. R., Guo, S., Cohen, P., and Untreman, T. G. (2001) Biochem. J. 354, 605–612
39. Cantó, C., Gerhart-Hines, Z., Feige, J. N., Lagouge, M., Noriega, L., Milne, J. C., Elliott, P. J., Puigserver, P., and Auwerx, J. (2009) Nature 458, 1056–1060