FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2$^{SIRT1}$

Armando van der Horst, Leon G.J. Tertoolen, Lydia M.M. de Vries-Smits, Roy A. Frye, René H. Medema, and Boudewijn M.T. Burgering

*The Journal of Biological Chemistry* **279**, 28873-28879 (2004)
FOXO transcription factors have important roles in metabolism, cellular proliferation, stress tolerance, and aging. FOXOs are negatively regulated by protein kinase Bc-Akt-mediated phosphorylation. Here we show that FOXO factors are also subject to regulation by reversible acetylation. We provide evidence that the acetyltransferase CREB-binding protein (CBP) binds FOXO resulting in acetylation of FOXO. This acetylation inhibits FOXO transcriptional activity. Binding of CBP and acetylation are induced after treatment of cells with peroxide stress. Deacetylation of FOXOs involves binding of the NAD-dependent deacetylase hSir2. Accordingly, hSir2-mediated deacetylation precludes FOXO inhibition through acetylation and thereby prolongs FOXO-dependent transcription of stress-regulating genes. These data demonstrate that acetylation functions in a second pathway of negative control for FOXO factors and provides a novel mechanism whereby hSir2 can promote cellular survival and increase lifespan.

The Forkhead box, class O subfamily of forkhead transcription factors (FOXO) contains the functionally related proteins FOXO1, FOXO3α, and FOXO4 (also known as FKHRL1, FKHR1, and AF4, respectively; Ref. 1). The growth factor-stimulated phosphatidylinositol 3-kinase-protein kinase B (PKB/Akt) pathway negatively regulates FOXO factors by phosphorylation-mediated nuclear exclusion (2–4). This pathway is evolutionarily conserved between Caenorhabditis elegans and humans. DAF-16, the C. elegans homologue of mammalian FOXO, is also controlled by phosphatidylinositol 3-kinase/PKB signaling. DAF-16 regulates dauer formation in C. elegans, of which the homologue of mammalian FOXO, is also controlled by phosphatidylinositol 3-kinase/PKB signaling. DAF-16 regulates dauer formation in C. elegans, of which the homologue of mammalian FOXO, is also controlled by phosphatidylinositol 3-kinase/PKB signaling. DAF-16 regulates dauer formation in C. elegans, of which the homologue of mammalian FOXO, is also controlled by phosphatidylinositol 3-kinase/PKB signaling.
sense sequence, CAAACUUGAGAAGAAGACCTGG; duplex 2, sense sequence, CGGUGAAGACCUCUGGUAACdTdT; duplex 3, sense sequence, CGUCCUGAUCGCUAGUCUCUdTdT) were purchased from Dharmaco RNA Technologies and cotransfected using Oligo-fectAMINE according to the manufacturer (Invitrogen).

GST-FOXO4-DB, GST-β300-HAT, and GST-tagged hSir2SIRT1 were purified from bacteria using a standard GST-fusion protein-purification protocol (23).

Antibodies—Monoclonal 12C5A and 9E10 antibodies were produced using a transfected cell line. Monoclonal antibodies recognizing the FLAG-M2 epitope, p27\(^{kip1}\), and GAPDH were obtained from Sigma, Transduction Laboratories, and Chemicon, respectively. Polyclonal antibodies recognizing acetylated lysine residues (ε-Lys), actin, CBP, Gal-DBD, MnSOD, and p53Δ7-9-pKB were obtained from Cell Signaling Technologies, Santa Cruz Biotechnology (3x), Stresgen BioReagents, and New England Biolabs, respectively. Polyclonal antibodies recognizing FOXO3a were purchased from Sigma and Santa Cruz Biotechnology. Polyclonal antibodies recognizing FOXO4 (19) and PKB (15) have been described before. Polyclonal antibody recognizing hSir2SIRT1 was raised in rabbit using an N terminally His-tagged fragment consisting of amino acids 506–747 of hSir2SIRT1.

Western Blot Analysis—Protein samples were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane (Immobilon). Western blot analysis was performed under standard conditions using the indicated antibodies.

**FOXO Activity**—To determine the expression of endogenous p27\(^{kip1}\) and MnSOD, HEK293T cells were transfected with empty vector or HA-FOXO4 together with pBabe-puro. Either constructs encoding pBabe-puro (23) or pBabe-puro-FOXO3a were purchased from Sigma and Santa Cruz Biotechnology, respectively. Sensitivity to hydrogen peroxide or Nam was determined in these cells. FOXO activity was detected using an antibody recognizing p27\(^{kip1}\) and/or the hSir2SIRT1 inhibitor. Western blot analysis was performed using hybridoma cell lines. Monoclonal antibodies recognizing the FLAG-M2 epitope, p27\(^{kip1}\), and GAPDH were obtained from Sigma, Transduction Laboratories, and Chemicon, respectively. Polyclonal antibodies recognizing acetylated lysine residues (ε-Lys), actin, CBP, Gal-DBD, MnSOD, and p53Δ7-9-pKB were obtained from Cell Signaling Technologies, Santa Cruz Biotechnology (3x), Stresgen BioReagents, and New England Biolabs, respectively. Polyclonal antibodies recognizing FOXO3a were purchased from Sigma and Santa Cruz Biotechnology. Polyclonal antibodies recognizing FOXO4 (19) and PKB (15) have been described before. Polyclonal antibody recognizing hSir2SIRT1 was raised in rabbit using an N terminally His-tagged fragment consisting of amino acids 506–747 of hSir2SIRT1.

**RESULTS**

Nicotinamide Inhibits FOXO Transcriptional Activity—To investigate the possibility that hSir2SIRT1 controls FOXO function, we analyzed the effect of Nam, an inhibitor of the Sir2 family of deacetylases (25), on FOXO transcriptional activity. Nam inhibited FOXO4-mediated transcription of a luciferase reporter construct bearing six canonical FOXO-binding sites (6C35) in HEK293T cells increased p27\(^{kip1}\) expression (Fig. 1C, left panel). In addition to imposing a cell-cycle arrest, FOXO3a activation increased MnSOD expression, and, similar to FOXO-induced cell-cycle arrest, FOXO activation increases cellular resistance to oxidative stress through transcriptional regulation of the gene encoding manganese superoxide dismutase (MnSOD) (10). Activation of FOXO in DL23 cells by 4-OHT treatment increased MnSOD expression, and, similar to FOXO-induced p27\(^{kip1}\) expression, MnSOD levels were reduced in cells transfected with Nam (Fig. 1C, left panel). In keeping with MnSOD mediating FOXO-induced cell-cycle arrest against oxidative stress, we could observe a similar effect in a mouse fibroblast cell line, NIH-3T3, that had been transfected with a reporter construct bearing six canonical FOXO-binding sites (6C35) and lacking functional p53, and demonstrated in these cells transcriptional regulation of p27\(^{kip1}\) expression and induction of a G1 cell-cycle arrest by FOXO (10). Treatment of DL23 cells with 4-OHT induced p27\(^{kip1}\) expression (Fig. 1C, left panel). This induction was reduced by treatment of these cells with Nam (Fig. 1C, left panel). Furthermore, FOXO3a activation imposed a cell-cycle arrest, which was partially relieved by treatment with Nam, in keeping with its effect on p27\(^{kip1}\) expression. In addition to imposing a cell-cycle arrest, FOXO activation increases cellular resistance against oxidative stress through transcriptional regulation of the gene encoding manganese superoxide dismutase (MnSOD) (10). Activation of FOXO in DL23 cells by 4-OHT treatment increased MnSOD expression, and, similar to FOXO-induced p27\(^{kip1}\) expression, MnSOD levels were reduced in cells transfected with Nam (Fig. 1C, left panel).
posttranslational modifications of Forkhead box O transcription factors

FIG. 1. Nam inhibits the transcriptional and biological activity of FOXO4. A, A14 cells were transfected with empty vector or a construct encoding HA-FOXO4 together with 6× DBE-luciferase and treated with Nam. All samples were assayed in triplicate. Luciferase counts were normalized using Tr-Renilla-luciferase (luciferase ratio), and the fold induction of luciferase expression was determined by dividing luciferase ratios from samples transfected with FOXO4 by the ratios from parallel samples transfected with empty vector to control for effects on basal transcription. *, p < 0.05. B, HEK293T cells were transfected with empty vector or a construct encoding HA-FOXO4 and treated with Nam. Lysates of transfected cells were assayed for p27kip1, HA-FOXO4, pS473-PKB, and PKB expression. GAPDH expression was used to monitor equal protein loading. C, DL23 cells were treated for twenty-four hours using Nam and/or 4-OHT. Total lysates were analyzed by Western blotting for the expression of p27kip1, MnSOD, and actin (left panel). The percentage of DL23 cells in S-phase was determined by flow cytometry of bromodeoxyuridine-stained cells. Histograms were analyzed using ModFit LT software. *, p < 0.05; **, p < 0.001. D, the kinetics of CM-H2DCF probe oxidation in DL23 cells (upper and lower left panels) and A14 cells (lower right panel) was measured after H2O2 (150 μM) addition (average of 4–5 cells/experiment). Curves were fitted with a single exponent according to $f(x) = e^{-kt}$, using $\chi^2$ minimization. All measurements had similar half-lives ($t = 172 ± 14$ s (left panel). The line representing the control + Nam was omitted from the graph for reasons of clarity (it was in between the control and 4-OHT + Nam lines). Steady-state oxidation levels were measured at 800 s (lower panels). *, p < 0.05. The experiments presented are representative of at least three independent experiments.

stress, H2O2-induced oxidative stress was reduced in cells treated with 4-OHT, whereas Nam treatment inhibited the effect of FOXO3a (Fig. 1D, upper and lower left panels). In addition, A14 cells expressing active FOXO3a after retroviral transduction displayed increased resistance against H2O2-induced oxidative stress, and this was also reversed by Nam treatment (Fig. 1D, lower right panel). These results show that inhibition of Sir2 function by Nam treatment inhibits FOXO-induced gene expression of p27kip1 and MnSOD and, consequently, FOXO-induced cell-cycle arrest and resistance against oxidative stress.

FIG. 2. hSir2SIRT1 enhances FOXO4 transcriptional and biological activity. A, HEK293T cells were transfected as described for Fig. 1B together with a myc-hSir2SIRT1 construct. p27kip1, MnSOD, and HA-FOXO4 expressions were examined by Western blotting. GAPDH expression was used to monitor equal loading. B, HEK293T cells were transfected with three different siRNA oligonucleotides and/or HA-FOXO4. Forty hours after transfection, cells were harvested and total lysates were assayed for p27kip1, MnSOD, hSir2SIRT1, and FOXO4 expression. GAPDH was used to check for equal loading. C, A14 cells were transfected with empty vector or HA-FOXO3a.A3 together with hSir2SIRT1. Cells were harvested forty hours posttransfection, and the increase in percentage of cells in the G1-phase of the cell cycle as compared with untransfected cells was determined by flow cytometry of propidium iodide-stained cells. Histograms were analyzed using ModFit LT software. *, p < 0.05. The experiments presented are representative of at least three independent experiments.
modulates its biological activity.

**CBP Acetylates FOXO and Inhibits FOXO Transcriptional Activity**—As Sir2 deacetylases are generally thought to act as repressors of transcription via deacetylating histones (26) we hypothesized that hSir2<sup>SIRT1</sup> directly deacetylates FOXO transcription factors. Acetylation of proteins is mediated by acetyltransferases such as the related proteins p300 and CBP; binding between p300/CBP and FOXO as well as FOXO acetylation have been reported (27–29). To confirm these findings, HA-CBP and FLAG-FOXO4 were co-expressed in HEK293T cells. Indeed FLAG-FOXO4 was found to bind to HA-CBP (Fig. 3A). More importantly, binding of CBP to FOXO4 induced acetylation of FOXO4 (Fig. 3B). Also, co-expression of CBP and FOXO4a resulted in acetylation of FOXO4a (data not shown), suggesting that CBP is able to mediate acetylation of other FOXO transcription factors. Similarly, co-expression of p300 and FOXO4 induced acetylation of FOXO4 (data not shown), indicating that CBP and p300 are functionally equivalent in FOXO4 acetylation.

Because inhibiting hSir2<sup>SIRT1</sup> activity by either Nam or siRNA-mediated knockdown of hSir2<sup>SIRT1</sup> inhibited transcriptional activity of FOXO, we next determined the effect of CBP-mediated acetylation of FOXO4 on its transcriptional activity. In A14-p27lac cells expressing wt-CBP, FOXO4 activity was considerably impaired as compared with control cells expressing FOXO4 only (Fig. 3D). This repression was due to the acetyltransferase activity of CBP as co-expression of the CBP-mutant F1541A, which possesses reduced but not absent acetyltransferase activity of CBP, or an acetyltransferase-impaired mutant (CBP-F1541A) inhibited the induction of p27<sup>kip1</sup> (Fig. 3D). This repression was due to the acetyltransferase activity of CBP as co-expression of the CBP-mutant F1541A, which possesses reduced but not absent acetyltransferase activity of CBP, or an acetyltransferase-impaired mutant (CBP-F1541A) inhibited the induction of p27<sup>kip1</sup> (Fig. 3D). Subsequently, bacterially expressed GST-hSir2<sup>SIRT1</sup> was added in the presence or absence of NAD⁺ for 90 min. Samples were assayed by Coomasie blue staining and autoradiography. G, HEK293T cells were transfected with constructs encoding myc-FOXO4, HA-CBP, and hSir2<sup>SIRT1</sup>. Immunoprecipitation samples and total lysates were analyzed by Western blotting using anti-acetylated lysine (AcLys), anti-myc (9E10), and anti-Gal4-DBD antibodies. The experiments presented are representative of at least three independent experiments.

**Immunoprecipitation**—Using Western blotting using anti-acetylated lysine (AcLys), anti-HA (12CA5), and anti-FLAG-M2 antibodies. C, HEK293T cells were cotransfected with constructs encoding HA-FOXO4, CBP and FLAG-FOXO4. CBP immunoprecipitates and total lysates were analyzed by Western blotting using anti-HA (12CA5) and anti-FLAG-M2 antibodies. The experiments presented are representative of at least three independent experiments.
Posttranslational modifications of Forkhead box O transcription factors

DB was in vitro acetylated by a GST-tagged p300 acetyltransferase domain in the presence of [14C]Acetyl-CoA (21). Subsequently, the reactions were incubated with GST-tagged hSir2shRTT in the absence or presence of the essential cofactor NAD+ (24). GST-hSir2shRTT completely deacetylated GST-FOXO4-DB in a NAD+-dependent manner (Fig. 3F). The incomplete inhibition of deacetylase activity by omitting NAD+ was possibly due to traces of NAD+ in the GST-hSir2shRTT purification. Next, we tested deacetylation by GST-FOXO4 was observed upon cotransfection with hSir2shRTT (Fig. 3G). Taken together, these results show that hSir2shRTT can directly deacetylate FOXO4 both in vitro and in vivo and thereby stimulate FOXO transcriptional and biological activity.

Hydrogen Peroxide Induces Acetylation of FOXO and Inhibits FOXO Activity—To investigate whether extracellular signaling could regulate acetylation/deacetylation of FOXO4, we tested the effects of insulin/insulin-like growth factor-1, a known regulator of FOXO function, and hydrogen peroxide. Hydrogen peroxide is known to promote acetylation of proteins like p53 and histones (31, 32), and FOXO transcription factors have been shown to be important in regulating stress responses (10). First, we investigated whether hydrogen peroxide could affect binding of CBP to FOXO4. Therefore, HA-CBP was immunoprecipitated from HEK293T cells co-expressing myc-FOXO4. Increasing amounts of hydrogen peroxide enhanced the interaction between HA-CBP and myc-FOXO4 (Fig. 4A), suggesting that peroxide stress may induce acetylation of FOXO4 by CBP. Furthermore, activation of phosphatidylinositol 3-kinase/PKB signaling by treating cells with insulin and epidermal growth factor increased the basal binding between HA-CBP and myc-FOXO4. As CBP is localized exclusively within the nucleus, the decreased binding observed is likely due to the fact that phosphatidylinositol 3-kinase/PKB signaling induces relocalization of myc-FOXO4 to the cytosol. Importantly, activation of phosphatidylinositol 3-kinase/PKB signaling could not prevent the increase of HA-CBP/myc-FOXO4 complex formation induced by hydrogen peroxide treatment, indicating that the effect of peroxide is dominant over growth factor stimulation in this respect. Next, we determined whether endogenous FOXO and CBP could interact. We detected endogenous interaction between CBP and both FOXO4 and FOXO3a. Similar to the transient expression studies, H2O2 treatment increased the interaction between FOXO and CBP, although the magnitude differed (Fig. 4B). Finally, we tested whether enhanced binding of CBP to FOXO4 after treatment of cells with hydrogen peroxide resulted in increased acetylation of FOXO4. Thus, HEK293T cells transfected with myc-FOXO4 construct were treated with different concentrations of peroxide and followed up for several periods of time. FOXO4 was immunoprecipitated and acetylation was determined by Western blotting. Hydrogen peroxide treatment indeed induced acetylation of FOXO4 (Fig. 4C). Also, treatment of cells cotransfected with FLAG-FOXO4 and HA-CBP with hydrogen peroxide resulted in increased acetylation in the fraction of FLAG-FOXO4 bound to HA-CBP (Fig. 4D). As noted, we detected acetylation by endogenous acetyltransferases only at later timepoints than would be suggested by the binding of CBP to FOXO4 (Fig. 4, A and B). As we used a pan-acetyllysine-antibody, we attribute this result to the low sensitivity of the antibody toward FOXO4, precluding detection of a low stoichiometry. This is further suggested by the observation that upon overexpression of CBP, we detected acetylation at shorter timepoints after peroxide treatment. Therefore, increased binding of FOXO4 to CBP after hydrogen peroxide treatment likely leads to increased acetylation.

As hydrogen peroxide induces acetylation of FOXO4, we expected that peroxide would have the same effect as CBP or Nam on FOXO4 transcriptional activity. To study this, FOXO4 was transfected in HEK293T cells, and the expression of endogenous p27kip1 was determined upon treatment of these cells with various concentrations of hydrogen peroxide for twenty-four hours. Indeed, hydrogen peroxide inhibited p27kip1 expression (Fig. 4E). Inhibition of p27kip1 expression was not due to activation of PKB by hydrogen peroxide, as under these conditions we could not detect any changes in the Ser-473 phosphorylation of PKB. Taken together, these data indicate that hydrogen peroxide inhibits FOXO4 transcriptional activity through induction of FOXO4 acetylation.

Hydrogen Peroxide Stimulates Binding of hSir2shRTT to FOXO—Finally, because acetylation of FOXO4 occurs in conjunction with the binding of the acetyltransferase CBP, we also investigated whether deacetylation by hSir2shRTT involves direct binding. Therefore, we expressed myc-hSir2shRTT, HA-CBP, and treated cells with Nam or hydrogen peroxide for one hour to induce acetylation of HA-FOXO4. Complex formation between hSir2shRTT and FOXO4 could only be detected after peroxide treatment (Fig. 4F), suggesting that hSir2shRTT binds to acetylated FOXO4. On the contrary, Nam was not able to stabilize the interaction of hSir2shRTT with FOXO4, indicating that the effect of Nam on FOXO transcriptional activity cannot be accounted for by mere binding of hSir2shRTT to FOXO. We also tested whether FOXO and hSir2shRTT interacted endogenously. Antibody quality precluded the detection of a clear interaction between FOXO4 and hSir2shRTT, but in HEK293T cells, an interaction between endogenous FOXO3a and hSir2shRTT was detected, and this interaction was weakly induced by peroxide treatment (Fig. 4G). Taken together, these data show that hSir2shRTT directly binds to FOXO, which is induced by peroxide stress.

**DISCUSSION**

Our results suggest a model in which FOXO transcription factors are subject to regulation by reversible acetylation. Acetylation of FOXO results in inhibition of their transcriptional and biological activities, which is reversed by the longevity protein hSir2shRTT. Inhibition through acetylation of FOXO transcription factors is functionally equivalent to the previously described inhibition by PKB-mediated phosphorylation.

We show that the acetyltransferase CBP binds to and acetylates FOXO4, thereby inhibiting its transcriptional activity. Previous reports on CBP/p300 binding to FOXO (27, 28) suggested that CBP/p300 binding is required for activation of signaling PKB. Taken together, these data show that CBP/p300 binding is required for activation of FOXO-dependent transcription. However, there are several important differences between these and our studies that may explain this apparent discrepancy. First, Nasrin et al. (28) inferred a role for CBP/p300 in the activation of FOXO because they observed that the viral protein E1A, which can inhibit CBP co-activator function, also inhibits FOXO transcriptional activity on an insulin-like growth factor BP1 promoter. However, E1A is known to have a plethora of targets within the transcriptional machinery and thus, by itself, a block by E1A is not sufficient proof of the involvement of CBP/p300 (for a review, see Ref. 33). Second, whereas we have also analyzed the effect of CBP on the regulation of endogenous gene transcription, other studies have relied on transient reporter assays in which a proper chromatin context is likely to be lacking. Third, the role of CBP/p300 can be promoter-context-dependent, and accordingly, different FOXO-responsive genes may respond differently to the presence of CBP/p300. Differential responsiveness could relate to the fact that we have to discriminate between the effect of CBP/p300 as histone acetyltransferase and as acetyltransferase acting on FOXO itself. It could be
Chapter 2: FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2\textsuperscript{SIRT1}

Inhibitory Acetylation of FOXO4 Is Reversed by hSir2\textsuperscript{SIRT1}

FIG. 4. Peroxide stress induces FOXO4 acetylation, thereby inhibiting FOXO4 transcriptional activity, and induces hSir2\textsuperscript{SIRT1} binding to FOXO. A, HEK293T cells were transfected with constructs encoding HA-CBP and myc-FOXO4. Cells were treated with the indicated concentrations of peroxide or insulin plus EGF for 30 min, and CBP was immunoprecipitated from cellular lysates. Two lanes at far right, insulin and EGF were given 30 min before adding peroxide. Immunoprecipitation samples and total lysates were analyzed by Western blotting using anti-HA (12CA5), anti-myc 9E10, and anti-pS473-PKB antibodies. B, C2C12 and HEK293T cells were treated for 1 h with 500 \( \mu \text{M} \) \( \text{H}_2\text{O}_2 \). Endogenous FOXOs (FOXO4 from C2C12 in upper panel, FOXO3a from HEK293T in lower panel) were immunoprecipitated, and immune complexes were assayed for the presence of CBP. Total lysates were also checked for CBP expression. C, HEK293T cells were transfected with a construct encoding myc-FOXO4 and treated with the indicated concentrations of hydrogen peroxide for 1 or 4 h. FOXO4 immunoprecipitation samples were analyzed by Western blotting using anti-acetyllysine (\( \alpha\)-AcLys) and anti-myc (9E10) antibodies. D, the experiment was performed essentially as in A. Immunoprecipitates were first subjected to anti-acetyllysine (\( \alpha\)-AcLys) antibody before probing for FOXO4. E, HEK293T cells were transfected as in Fig. 1B and treated with peroxide. \( \text{p27}^{\text{kip1}} \), HA-FOXO4, pS473-PKB, and PKB expression were examined by Western blotting. GAPDH expression was used to monitor equal loading. F, HEK293T cells were transfected with constructs encoding myc-hSir2\textsuperscript{SIRT1} and HA-FOXO4. Cells were treated with 200 \( \mu \text{M} \) hydrogen peroxide or 5 \( \mu \text{M} \) Nam. Either HA-FOXO4 (left panel) or myc-hSir2\textsuperscript{SIRT1} (right panel) was immunoprecipitated from cellular lysates. Immunoprecipitation samples and total lysates were analyzed by Western blotting using anti-HA (12CA5) and anti-myc (9E10) antibodies. G, HEK293T cells were treated for 1 h with 500 \( \mu \text{M} \) \( \text{H}_2\text{O}_2 \). Endogenous FOXO3a (left panel) or hSir2\textsuperscript{SIRT1} (right panel) were immunoprecipitated, and immune complexes were assayed for the presence of hSir2\textsuperscript{SIRT1} and FOXO3a, respectively. Total lysates were also checked for hSir2\textsuperscript{SIRT1} and FOXO3a expression, respectively. The experiments presented are representative of at least three independent experiments.
and consequently deacetylate FOXO4. Thus, hSir2SIRT1 can counteract the inhibition of FOXO induced by acetylation. Recently, regulation of p53 acetylation by hSir2SIRT1 has been described (16, 24). Furthermore, in cells lacking functional p53, resveratrol, a presumed activator of hSir2SIRT1, can still enhance protection against genotoxic stress (14). These observations suggest alternative p53-independent pathways involved in cellular protection by hSir2SIRT1. Recently, we have shown that FOXO activation results in increased resistance against oxidative stress (10), and here we show that this is regulated by hSir2SIRT1. We like to propose that FOXOs play an important role in the hSir2SIRT1-mediated, p53-independent protection against stress. Therefore, by preventing negative regulation of FOXO due to acetylation after increased oxidative stress, hSir2SIRT1 can enhance cellular defenses against oxidative stress provided for by FOXO. This may be especially relevant under conditions of endogenous oxidative stress occurring during normal metabolic activities. Oxidative stress is considered to be a prime parameter of aging in all living organisms. Therefore, the results presented in this report provide a rationale for the observed increase in longevity induced by C. elegans Sir2, which is FOXO-dependent.

Acknowledgments—We thank Hans Bos, Leo Price, Arjan Bremkman, and Bas Winkler for critical reading of the manuscript and stimulating discussions, Anneke Steegh for technical assistance, and Rachel Giles, Robert Vrees, and Eric Kalkhoven for providing DNA constructs and reagents.

REFERENCES

1. Kaestner, K. H., Knochel, W., and Martinez, D. E. (2000) Genes Dev. 14, 142–146
2. Brunet, A., Bonni, A., Zigmond, M. J., Lin, M. Z., Joo, P., Hu, L. S., Anderson, M. J., Arden, K. C., Bleas, J., and Greenberg, M. E. (1999) Cell 96, 857–866
3. Kops, G. J., de Ruiter, N. D., De Vries-Smits, A. M., Powell, D. R., Bos, J. L., and Burgers, R. M. (1999) Nature 398, 620–624
4. Tang, E. D., Nunez, G., Barr, F. G., and Guan, K. L. (1999) J. Biol. Chem. 274, 10741–10748
5. Gottlieb, S., and Bockman, G. (1994) Genetics 137, 107–123
6. Kenyon, C., Chung, J., Gems, E., Rudner, A., and Tart sb, R. (1993) Nature 366, 461–464
7. Murakami, S., and Johnson, T. E. (1996) Genetics 143, 1207–1218
8. Honda, Y., and Honda, S. (1999) PASE J. 13, 1385–1393
9. Tran, H., Brunet, A., Grenier, J. M., Datta, S. R., Furnace, A. J., Di Stefano, P. S., Chiang, L. W., and Greenberg, M. E. (2002) Science 296, 530–534
10. Kops, G. J., Dassen, T. B., Polderman, P. E., Saarloos, I., Wirtz, K. W., Coffer, P. J., Huang, T. T., Bos, J. L., Medema, R. H., and Burgers, R. M. (2002) Nature 419, 316–321
11. Burgers, R. M., and Kops, G. J. (2002) Trends Biochem. Sci 27, 352–360
12. Tissenbaum, H. A., and Guarente, L. (2001) Nature 416, 227–230
13. Frye, R. A. (2000) Biochem. Biophys. Res. Commun. 273, 783–788
14. Hovitz, K. R., Bitterman, K. J., Cohen, H. Y., Latorre-Esteves, M., and Greenberg, M. E. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 6283–6288
15. Ogg, S., Calvillo, D. G., Schubert, D., and Weinberg, R. A. (2001) Science 293, 301–305
16. Bitterman, K. J., and Greenberg, M. E. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 6283–6288
17. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
18. Furuyama, T., Nakazawa, T., Nakano, I., and Mori, N. (2000) Biochem. J. 349, 629–634
19. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
20. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
21. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
22. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
23. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
24. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
25. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
26. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
27. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
28. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
29. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
30. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
31. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
32. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
33. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
34. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
35. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
36. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
37. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
38. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
39. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533
40. Veech, R. L., and Sinha, S. (2002) Am. J. Clin. Nutr. 76, 1522–1533