Jefferson.

Thomas Jefferson University
Jefferson Digital Commons

Department of Cancer Biology Faculty Papers | Department of Cancer Biology

December 2007

Acetylation in nuclear receptor signaling and the role of sirtuins

Chenguang Wang
Thomas Jefferson University

Michael J. Powell
Thomas Jefferson University

Vladimir M. Popov
Thomas Jefferson University

Richard G. Pestell
Thomas Jefferson University, Cecilia.Deemer@kimmelcancercenter.org

Let us know how access to this document benefits you

Follow this and additional works at: http://jdc.jefferson.edu/cbfp

Part of the Amino Acids, Peptides, and Proteins Commons

Recommended Citation

Wang, Chenguang; Powell, Michael J.; Popov, Vladimir M.; and Pestell, Richard G., "Acetylation in nuclear receptor signaling and the role of sirtuins" (2007). *Department of Cancer Biology Faculty Papers.* Paper 11.
http://jdc.jefferson.edu/cbfp/11

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's Academic & Instructional Support & Resources Department (AISR). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Cancer Biology Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.

Mini Review in Molecular Endocrinology

Acetylation in Nuclear Receptor Signaling and the Role of Sirtuins

Authors: Chenguang Wang[1], Michael J. Powell[1], Vladimir M. Popov[1], and Richard G. Pestell[1,2]

Departments of Cancer Biology[1] & Medical Oncology[2]
Kimmel Cancer Center
Thomas Jefferson University
233 S. 10th Street
Philadelphia, PA 19107

Corresponding Author: Richard G. Pestell, M.D., Ph.D.
Department of Cancer Biology
Thomas Jefferson University
233 S. 10th Street
Philadelphia, PA 19107
Email: Richard.Pestell@jefferson.edu

DISCLOSURE STATEMENT: The authors have nothing to disclose.

Copyright (C) 2007 by The Endocrine Society

Abstract

It has been known since the early 1970s that nuclear receptor complexes bind DNA in association with co-regulatory proteins. Characterization of these nuclear receptor coregulators has revealed diverse enzymatic activities which temporally and spatially coordinate nuclear receptor activity within the context of local chromatin in response to diverse hormone signals. Chromatin modifying proteins, which dictate the higher order chromatin structure in which DNA is packaged, in turn orchestrate orderly recruitment of nuclear receptor complexes. Modifications of histones include acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, ADP ribosylation, deimination and proline isomerization (1). At this time, we understand how a subset of these modifications regulates nuclear receptor signaling. However the effects, particularly of acetylation and demethylation, are profound. The finding that nuclear receptors are directly acetylated and that acetylation in turn directly regulates contact-independent growth has broad therapeutic implications. Studies over the past 7 years have led to the understanding that nuclear receptor acetylation is a conserved function, regulating diverse nuclear receptor activity. Furthermore we now know that acetylation of multiple and distinct substrates within nuclear receptor signaling pathways, form an acetylation signaling network from the cell surface to the nucleus. The finding that NAD-dependent histone deacetylases, the sirtuins, are capable of deacetylating nuclear receptors provides a new level of complexity in the control of nuclear receptor activity in which local intracellular concentrations of NAD may regulate nuclear receptor physiology.

Nuclear Receptors Co-regulator Enzyme Complexes

In the early 1970s initial attempts at purifying nuclear receptors were confounded by the large number of co-associated proteins. The O'Malley laboratory had characterized the nuclear progesterone receptor/DNA complex and the thyroid hormone receptor associated with a heterogenous group of proteins that was regulated in a ligand-dependent manner (2, 3). It was apparent that transcription factors contained transactivation domains which functioned as modular surfaces to regulate transcription independently of direct binding to DNA (4). The laboratory of Tjian and others characterized the TATA box binding protein-associated factors termed TAFs (5). Several cell-type specific activities were characterized and shown to regulate transcription factor activity. In this regard a B cell-specific activity designated Oct coactivator from B cells (OCA-B) regulated Oct-dependent B cell specific transcription (6).

Cross squelching experiments by the Chambon laboratory (7) suggested distinct classes of transcriptional activation domains existed within nuclear receptors. Consistent with the notion that nuclear receptors were capable of repressing transcription, formal evidence that nuclear receptors contain specific repression domains was provided by studies of the progesterone receptor and retinoic acid receptor (8, 9). These studies provided the rational basis for the identification of proteins mediating transcriptional activation and repression of nuclear receptors. Yamamoto identified the SWI protein as a key activator of the glucocorticoid receptor in yeast (10). In 1994, CBP was cloned as a coactivator of CREB (11) and p300 as an E1A-interacting protein (12, 13). Of fundamental importance was the identification of histone acetyltransferase

enzymatic activity within the p300 activation domain. These proteins were shown to function as rate-limiting co-activators of nuclear receptor activity partially dependent upon their intrinsic histone acetyltransferase activity.

A dynamic and rapidly evolving field has characterized diverse types of enzymes (14). Furthermore, the assembly of these enzymes was shown to be temporally coordinated. The histone acetyltransferase, p300, enhanced the efficiency of transcriptional initiation from an estrogen-regulated template assembled within chromatin. The reassembly of active complexes during subsequent rounds of reinitiation did not require p300 (14). Indeed consistent with these findings, chromatin immunoprecipitation experiments identified temporarily coordinated multi-protein complexes associated with ERα and with endogenous ERα DNA binding sites. These studies showed coactivators were recruited in a cyclical manner in association with local chromatin. p300 was recruited to the promoter region of the ERα-responsive genes in the first phase of ERα binding, but not in subsequent cycles of ERα recruitment (15).

Nuclear receptor acetylation governs cellular growth potential

Histone acetyltransferases have been shown to acetylate diverse substrates. The first evidence that nuclear receptors served as direct substrates for histone acetyltransferases were studies by Fu et al. (16). The residues of AR acetylated by p300 *in vitro* were conserved between species. Point substitution mutations of the acetylation sites identified *in vitro* regulated ligand-dependent transactivation. Subsequent studies demonstrated that the nuclear receptor acetylation site is conserved between a subset of nuclear receptors, including the ERα, TRβ (17), PR and the GR

(18). With each of the nuclear receptors characterized to date, the acetylation sites regulate a subset of nuclear receptor functions with the AR currently being the best characterized. The addition of ligand, dihydrotestosterone, or other agonists such as bombesin, enhances androgen receptor acetylation (19). When reintroduced into AR-deficient human prostate cancer cells, gain of function point substitution of the AR acetylation site resulted in receptors that promote prostate tumor growth, both *in vitro* and *in vivo* (20). Characterization of the mechanism by which the androgen receptor acetylation site regulated contact-independent growth, indicated both enhancement of cellular proliferation and a reduction in cellular apoptosis (20, 21). The charge of lysine residues in the AR acetylation site regulated recruitment of p300, and in a reciprocal manner, disengagement of corepressor complexes including NCoR, HDAC, and Smad 3 (20).

The AR acetylation site regulates prostate cellular apoptosis. The Jun kinase kinase signaling pathway (MEKK1) induces cellular apoptosis in the presence of the AR. The AR acetylation site governs TRAIL-induced cellular apoptosis (21). Cells transduced with AR acetylation site gain-of-function mutants enhanced proliferation, associated with the induction of cell cycle markers such as Ki67 and cyclin D1. The relative abundance of cyclin D1 was increased in cells transduced with the AR activating mutants.

Chromatin immunoprecipitation analysis demonstrated that in addition to the enhanced recruitment of the AR to an androgen-responsive element binding site (ARE), the histone deacetylase inhibitor, TSA, enhanced AR recruitment in an AR acetylation site-dependent manner (22). AR acetylation site dead mutants showed reduced recruitment to the PSA promoter androgen-responsive element and reduced TSA-dependent recruitment. This suggests that the

acetylation site of the nuclear receptor regulated access in the context of local chromatin (22).

The ERα was shown to be acetylated at the same motif as the AR (23). Deletion analysis identified the primary site acetylated by p300 and a minimal peptide sufficient to serve as a substrate for p300 was characterized. The peptide encoding the ERα acetylation site conveyed similar substate specificity as histone H3. Proteomic analysis including LC/MS and Edman degradation sequencing, demonstrated K302 and K303 were preferential sites of acetylation. Lys K303 is frequently mutated in human breast cancer (24). The ERα acetylation mutants function as hyperactive mutants in the presence of low concentrations on its ligand estradiol (23, 24). The ERα coactivator SRC1 is acetylated, and point mutation of the SRC1 acetylation site results in an ACTR hyperactive mutant that fails to be transcriptionally down regulated (25). As mutation of the ERα and the ERα coactivator ACTR acetylation site, enhances ligand-dependent acetylation may serve to repress hormone signaling by recruiting co-repressor activity as shown for PGC1α (26), or through docking others as-yet-to-be-defined complexes. Consistent with this model ERα repression by BRCA1 (27, 28) is abolished by mutation of the ERα K302/303 acetylation site (RGP. CW unpublished data). Like PGC1α and p53, in which multiple acetylation sites have been identified (29), ERα has recently been shown to be acetylated at the additional residue K266/K268 (30). In these studies, SRC1/p300 was used as the source of HAT. The relative importance of the K302/303 vs. the K266/K268 site in the diverse physiological roles of the ERα remains to be determined.

A body of evidence has contributed to the current understanding that PGC1α, a transcriptional co-activator of nuclear receptors, is acetylated and the acetylation of PGC1α

regulates cellular metabolism. The metabolic coactivator PGC1α modulates the gluconeogenic pathway in fasting and diabetic states through interaction with several transcription factors (31, 32). PGC1α forms part of a multi-protein complex that includes the TRAP/Mediator complex, including TRAP220 binding to its C-Terminus (33). The endogenous PGC1α complex includes the histone acetyltransferase GCN5. p300 and SRC1 bind the N-terminal activation domain of PGC1α (34). PGC1α is directly acetylated by GCN5 resulting in a transcriptionally inactive protein which relocalizes from promoter regions to nuclear foci (26).

Acetylation of components within hormone signaling pathways

The addition of liganded nuclear receptor to a cell induces multiple downstream signaling cascades. A growing body of evidence has demonstrated that several additional components which regulate nuclear receptor signaling pathways are themselves substrates for histone acetyltransferases (Fig. 1). Additional components of nuclear receptor signaling cascade that are directly acetylated include MEKK1, Hsp90 (35, 36), and IKK (37). The acetylation of these nuclear receptor binding proteins, in turn, affects nuclear receptor function. Thus, for example, Hsp90-mediated glucocortical receptor (GR) maturation is regulated by acetylation of Hsp90 (36). Within the nucleus, components of the nuclear receptor coactivator or corepressor complexes are themselves substrates for histone acetyltransferases. Thus ACTR and MTA1 are histone acetylases, and ACTR and PGC-1α serve as substrates for histone acetyltransferases (25, 26). Other transcription factors known to work in *trans* to regulate nuclear receptor signaling, such as the forked protein, are acetylated, providing an additional level of complexity for cross talk between (FKHR) and ERα signaling (38).

The NAD-dependent histone deacetylases regulate hormone signaling

The histone deacetylases are divided into three groups. The Class 1 and 2 HDACs share homology to the yeast Rpd3p and Hda1p proteins. The Class 3 HDACs are homologus to the yeast transcriptional repressor Sir2p. The Class 3 HDACs are nicotinamide adenine dinucleotide (NAD)-dependent. Mammalian Sirtuins are conserved with 7 genes homologous to the yeast *Sir2* gene. The nuclear sirtuins (SIRT1, SIRT6 and SIRT7), the mitochondrial sirtuin (SIRT3, SIRT4 and SIRT5) and cytosolic (SIRT2) regulate diverse metabolic function. The distinct substrates and subcellular localization of the mammalian Sirtuins suggests significant diversity of function (39, 40).

SIRT1 has been characterized in its interactions with nuclear receptors and nuclear receptor coactivators. The functional interactions between SIRT1 and nuclear receptor signaling has been examined for the cointegrators p300 (41) and PGC1α (42-44), and for the nuclear receptor AR (45), PPARγ (46) and ERα (47). SIRT1 has been shown to deacetylate coactivators PGC1α and p300 and the nuclear receptors ERα and AR. SIRT1 regulates repression of the co-activator p300, providing a mechanism by which the NAD-dependent HAT deacetylases may regulate diverse nuclear receptor functions (41). The p300 coactivator functions as a rate-limiting protein in regulating diverse nuclear receptor functions. A direct link between the NAD-dependent histone deacetylases and the histone acetyltransferases was first demonstrated by Bouras *et al* (36) (Fig. 1). SIRT1 repressed p300 in an NAD-dependent manner. The lysine residues within the Cell Cycle Regulatory Domain (CRD1) of p300 were essential for SIRT1-mediated repression. Importantly Bouras *et al* demonstrated that lysine residues within p300

function as substrates for SIRT2 (41). SIRT1 also functions as a substrate for sumoylation (41). SSP3, a sumo specific protease, antagonized SIRT1-mediated repression of p300. Importantly these studies suggest a link between local metabolic changes which are anticipated to alter the NAD/NADH ratio and p300 activity. As sirtuins are regulated by NAD, endogenous levels of nicotinamide may limit SIRT1 function and thereby alter activity of many transcription factors and nuclear receptors which are regulated by p300.

Sirtuins have well characterized functions in regulating responses to caloric restriction and glucose metabolism, cellular growth and DNA repair. SIRT1 regulates gluconeogenesis by promoting the repression of glycolytic gene function (40). Importantly, recent studies have linked SIRT1-dependent deacetylation of the nuclear receptor coactivator PGC1α to glucose homeostasis. PGC1α is acetylated *in vivo (43).* Acetylation of PGC1α was augmented by treatment with the SIRT1 inhibitor nicotinamide or by expression of the transcriptional coactivator p300 (28). The finding that SIRT1 associates with PGC1α (28) led to studies examining the biological significance of PGC1α acetylation. Fasting was shown to induce PGC1α deacetylation in skeletal muscle. This important study, identifying SIRT1 as a functional regulator of PGC1α to induce mitochondrial fatty acid oxidation *in vivo,* has broad implications for the understanding of adaptations to altered availability of nutrients. SIRT1 is increased in the fasted liver, thereby deacetylating and activating PGC1α, enhancing hepatic glucose output (43).

These studies imply that PGC1α abundance may in turn govern metabolic diseases such as obesity and diabetes. Recent studies using Resveratrol (RSV), a known activator of SIRT1 (48), demonstrated an increased aerobic capacity and induction of oxidative phosphorylation and mitochondrial biogenesis, primarily mediated by RSV-mediated inhibition of PGC1α acetylation

and an increase in PGC1α activity (49). Resveratrol was also shown to oppose the effects of a high calorie diet producing chances associated with longer lifespan (50). Caloric restruction enhances SIRT1 binding to and repression of PPARγ-responsive gene promoters. SIRT1 repressed PPARγ activity through binding the co-repressors NCoR and SMRT (46). Consistent with the physiological significance of SIRT1-mediated repression of PPARγ activity, *SIRT1*$^{+/-}$ mice demonstrated defective mobilization of fatty acids from white adipocytes upon fasting (46). Whether PPARγ or components within a PPARγ complex are direct substrates of acetylation, remains to be determined. Collectively, these studies demonstrate the important physiological role for PGC1α acetylation and a key role for the NAD-dependent histone deacetylase SIRT1 in regulating fat metabolism.

Recent studies have provided indirect evidence for an additional potential functional interaction between nuclear receptors and the sirtuins. Studies from the laboratories of Rosenfeld and Glass demonstrated the induction of transient double-stranded DNA breaks upon the addition of estradiol, and the recruitment of ERα, PARP-1 and DNA topoisomerase IIβ (51). The *BRCA1* gene which plays an essential role in DNA repair, is known to bind the ERα in the context of local chromatin in a temporally coordinated manner (28). Although the role of ERα acetylation in recruitment to double-stranded DNA breaks is unknown, Sirtuins, in particular SIRT6, regulate DS-DNA break repair. SIRT6 plays a key role in resistance to DNA damage and suppression of genomic instability (52). The fibroblasts of *SIRT1*$^{-/-}$ mice showed enhanced sensitivity to DNA damaging agents. Although the sirtuins and the ERα have both been independently implicated in DS-DNA break repair, it remains to be determined with sirtuins regulate this nuclear receptor function.

The use of Sirt1 inhibitors has strongly implicated endogenous SIRT1 in regulating AR activity in prostate cancer cells (37). SIRT1 colocalizes with the AR in nuclear subdomains (37). SIRT1 inhibits cellular proliferation in an AR-dependent manner. SIRT1 antagonists induce endogenous AR expression and enhance DHT-mediated AR-dependent gene expression. SIRT1 physically binds to and deacetylates the AR at a conserved lysine motif. The AR served as a substrate for the SIRT1 orthologs of several species including the mouse mSir2a, hSirt1 and two other enzymes *Cerevisiae* (SIR2p) and *Archaeoglobus fulgidus* (Sir2af2). Each of these enzymes were examined for deacetylation activity against a synthetic AR peptide known to be acetylated by p300. The deacetylation of the AR by the Sirtuins of several species was characterized by LCMS and MSMS fragmentation analysis. Thus the AR is a deacetylated by SIRT1 to inhibit ligand-dependent AR activity to thereby inhibit DHT-dependent prostate cancer cellular proliferation.

What might be the physiological role of Sirtuin dependent regulation of nuclear receptor function? Sirtuin expression and activity is itself under complex control and may impact nuclear receptor function. The importance of NAD in Sir2 activity implies Sir2 activity may be regulated by intracellular NAD, by the NAD/NADH ratio, or nicotinamide (53, 54). Elevated lactate, which reduces the NAD/NADH ratio, is predicted to inhibit SIRT1 (55). Inhibition of SIRT1 may enhance AR function (45) with consequent anabolic repair effects within muscle (56, 57). During prostate cancer progression a shift towards cytosolic glycolysis occurs (58, 59). Increased local lactate concentration correlates with prostate cancer progression (60, 61), which may be predicted to inhibit SIRT activity and activate the AR (45). Sirtuins are located in diverse subcellular locations and it will be of interest to determine whether receptors in non-nuclear

compartments are regulated by Sirtuins.

Conclusion

Nuclear receptors serve as substrates for Sirtuins and play a role in glucose metabolism, cell growth and DNA-repair. Evidence suggests sirtuins regulate nuclear receptors and nuclear receptor coactivators to coordinate diverse physiological functions. Histones are modified by at least eight distinct types of enzyme. Post-translational modification of histones by specific enzymes determines their subsequent types of enzymatic modification. Acetylation of lysine residues provides docking sites for other enzymes which coordinate sequential enzymatic reactions. Distinct modifications of lysine residues are mutually exclusive. It has been proposed that post-translational modification of histones imparts specific genetic output and signaling specificity. Thus, post-translational modification of lysines within histone tails results in specific types of signaling. Nuclear receptors undergo acetylation, phosphorylation, sumoylation and methylation. It is likely that these post-translational modifications are part of a similar coordinated signaling cascade that occurs within the receptor. The mechanisms by which post-translational modification within nuclear receptors contributes to the specificity of hormone signaling has broad implications for health and disease, in particular hormone-responsive cancers.

REFERENCES

1. Kouzarides, T. Chromatin modifications and their function. Cell, *128:* 693-705, 2007.
2. Spelsberg, T. C., Steggles, A. W., and O'Malley, B. W. Progesterone-binding components of chick oviduct. Chromatin acceptor sites. J. Biol. Chem., *246:* 4188-4197, 1971.
3. Murray, M. B. and Towle, H. C. Identification of nuclear factors that enhance binding of the thyroid hormone receptor to a thyroid hormone response element. Mol Endocrinol, *3:* 1434-1442, 1989.

4. Ma, J. and Ptashne, M. Converting a eukaryotic transcriptional inhibitor into an activator. Cell, *55*, 1988.
5. Dynlacht, B. D., Hoey, T., and Tjian, R. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell, *66:* 563576, 1991.
6. Luo, R. X., Postigo, A. A., and Dean, D. C. Rb interacts with histone deacetylase to repress transcription. Cell, *92:* 463-473, 1998.
7. Tasset, D., Tora, L., Fromental, C., Scheer, E., and Chambon, P. Distinct classes of transcriptional activating domains function by different mechanisms. Cell, *62:* 1177-1187, 1990.
8. Vegeto, E., Allan, G.F., Schrader, W.T., Tsai, M.J., McDonnell, D.P., O'Malley, B.W., The mechanism of RU486 antagonism is dependent on the conformation of the carboxyterminal tail of the human progesterone receptor. Cell, *69:* 703-713, 1992.
9. Allan, G. F., Leng, X., Tsai, S. Y., Weigel, N. L., Edwards, D. P., Tsai, M. J., and O'Malley, B. W. Hormone and antihormone induce distinct conformational changes which are central to steroid receptor activation. J. Biol Chem, *267:* 19513-19520, 1992.
10. Yoshinaga, S. K., Peterson, C. L., Herskowitz, I., and Yamamoto, K. R. Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science, *258:* 1598-1604, 1992.
11. Kwok, R. P. S., Lundblad, J. R., Chrivia, J. C., Richards, J. P., Bachinger, H. P., Brennan, R. G., Roberts, S. G. E., Green, M. R., and Goodman, R. H. Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature, *370:* 223226, 1994.
12. Yee, S. P. and Branton, P. E. Detection of cellular proteins associated with human adenovirus type 5 early region 1A polypeptides. Virology, *147:* 142-153, 1985.
13. Harlow, E., Whyte, P., Franza, B. R., Jr., and Schley, C. Association of adenovirus early-region 1A proteins with cellular polypeptides. Mol Cell Biol, *6:* 1579-1589, 1986.
14. Kraus, W. L. and Kadonaga, J. T. p300 and estrogen receptor cooperatively activate transcription via differential enhancement of intiation and reinitiation. Genes and Dev., *12:* 331-342, 1998.
15. Shang, Y., Hu, X., DiRenzo, J., Lazar, M. A., and Brown, M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell, *103:* 843-852, 2000.
16. Fu, M., Wang, C., Reutens, A. T., Wang, J., Angeletti, R. H., Siconolfi-Baez, L., Ogryzko, V., Avantaggiati, M. L., and Pestell, R. G. p300 and p300/cAMPresponse element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. J Biol Chem, *275:* 20853-20860, 2000.
17. Lin, H. Y., Hopkins, R., Cao, H. J., Tang, H. Y., Alexander, C., Davis, F. B., and Davis, P. J. Acetylation of nuclear hormone receptor superfamily members: thyroid hormone causes acetylation of its own receptor by a mitogen-activated protein kinase-dependent mechanism. Steroids, *70:* 444-449, 2005.
18. Ito, K., Yamamura, S., Essilfie-Quaye, S., Cosio, B., Ito, M., Barnes, P. J., and Adcock, I. M. Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-kappaB suppression. J Exp Med, *203:* 7-13, 2006.
19. Gong, J., Zhu, J., Goodman, O. B., Pestell, R. G., Schlegel, P. N., Nanus, D. M., and Shen, R. Activation of p300 histone acetyltransferase activity and acetylation of the androgen receptor by bombesin in prostate cancer cells. Oncogene, *25:* 2011-2021, 2006.
20. Fu, M., Wang, C., Wang, J., Sakamaki, T., Di Vizio, D., Zhang, X., Albanese, C., Balk, S., Chang, C., Fan, S., Rosen, E., Palvimo, J. J., Janne, O. A., Muratoglu, S., Avantaggiati, M., and Pestell , R. G. Acetylation of the androgen receptor enhances coactivator binding and promotes prostate cancer cell growth. Mol Cell Biol, *23:* 8563-

8575, 2003.
21. Fu, M., Wang, C., Wang, J., Sakamaki, T., Zhang, X., Yeung, Y.-G., Chang, C., Hopp, T., Fuqua, S. A. W., Jaffray, E., Hay, R. T., Palvimo, J. J., Jänne, O. A., and Pestell, R. G. The Androgen Receptor Acetylation governs transactivation and MEKK1-induced apoptosis without affecting in vitro sumoylation and transrepression function. Mol. Cell. Biol., *22:* 3373-3388, 2002.
22. Fu, M., Rao, M., Wu, K., Wang, C., Zhang, X., Hessien, M., Yeung, Y. G., Gioeli, D., Weber, M. J., and Pestell, R. G. The androgen receptor acetylation site regulates cAMP and AKT but not ERK-induced activity. J Biol Chem, *279:* 29436-29449, 2004.
23. Wang, C., Fu, M., Angeletti, R. H., Siconolfi-Baez, L., Reutens, A. T., Albanese, C., Lisanti, M. P., Katzenellenbogen, B. S., Kato, S., Hopp, T., Fuqua, S. A., Lopez, G. N., Kushner, P. J., and Pestell, R. G. Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity. J Biol Chem, *276:* 18375-18383, 2001.
24. Fuqua, S. A., Wiltschke, C., Zhang, Q. X., Borg, A., Castles, C. G., Friedrichs, W. E., Hopp, T., Hilsenbeck, S., Mohsin, S., O'Connell, P., and Allred, D. C. A hypersensitive estrogen receptor-alpha mutation in premalignant breast lesions. Cancer Res, *60:* 4026-4029, 2000.
25. Chen, H., Lin, R. J., Xie, W., Wilpitz, D., and Evans, R. M. Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. Cell, *98:* 675-686, 1999.
26. Lerin, C., Rodgers, J. T., Kalume, D. E., Kim, S. H., Pandey, A., and Puigserver, P. GCN5 acetyltransferase complex controls glucose metabolism through transcriptional repression of PGC-1alpha. Cell Metab, *3:* 429-438, 2006.
27. Fan, S., Wang, J., Yuan, R., Ma, Y., Meng, Q., Erdos, M. R., Pestell, R. G., Yuan, F., Auborn, K. J., Goldberg, I. D., and Rosen, E. M. BRCA1 inhibition of estrogen receptor signaling in transfected cells. Science, *284:* 1354-1356, 1999.
28. Wang, C., Fan, S., Li, Z., Fu, M., Rao, M., Ma, Y., Lisanti, M. P., Albanese, C., Katzenellenbogen, B. S., Kushner, P. J., Weber, B., Rosen, E. M., and Pestell, R. G. Cyclin D1 antagonizes BRCA1 repression of estrogen receptor alpha activity. Cancer Res, *65:* 6557-6567, 2005.
29. Knights, C. D., Catania, J., Giovanni, S. D., Muratoglu, S., Perez, R., Swartzbeck, A., Quong, A. A., Zhang, X., Beerman, T., Pestell, R. G., and Avantaggiati, M. L. Distinct p53 acetylation cassettes differentially influence gene-expression patterns and cell fate. J Cell Biol, *173:* 533-544, 2006.
30. Kim, M. Y., Woo, E. M., Chong, Y. T. E., Homenko, D. R., and Kraus, W. L. Acetylation of estrogen receptor a by p300 at lysines 266 and 268 enhances the deoxyribonucleic acid binding and transactivation activies of the receptor. Molecular Endocrinology, *20:* 1479-1493, 2006.
31. Yoon, J. C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelmant, G., Stafford, J., Kahn, C. R., Granner, D. K., Newgard, C. B., and Spiegelman, B. M. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature, *413:* 131-138, 2001.
32. Knutti, D. and Kralli, A. PGC-1, a versatile coactivator. Trends Endocrinol Metab, *12:* 360-365, 2001.
33. Wallberg, A. E., Yamamura, S., Malik, S., Spiegelman, B. M., and Roeder, R. G. Coordination of p300-mediated chromatin remodeling and TRAP/mediator function through coactivator PGC-1alpha. Mol Cell, *12:* 1137-1149, 2003.

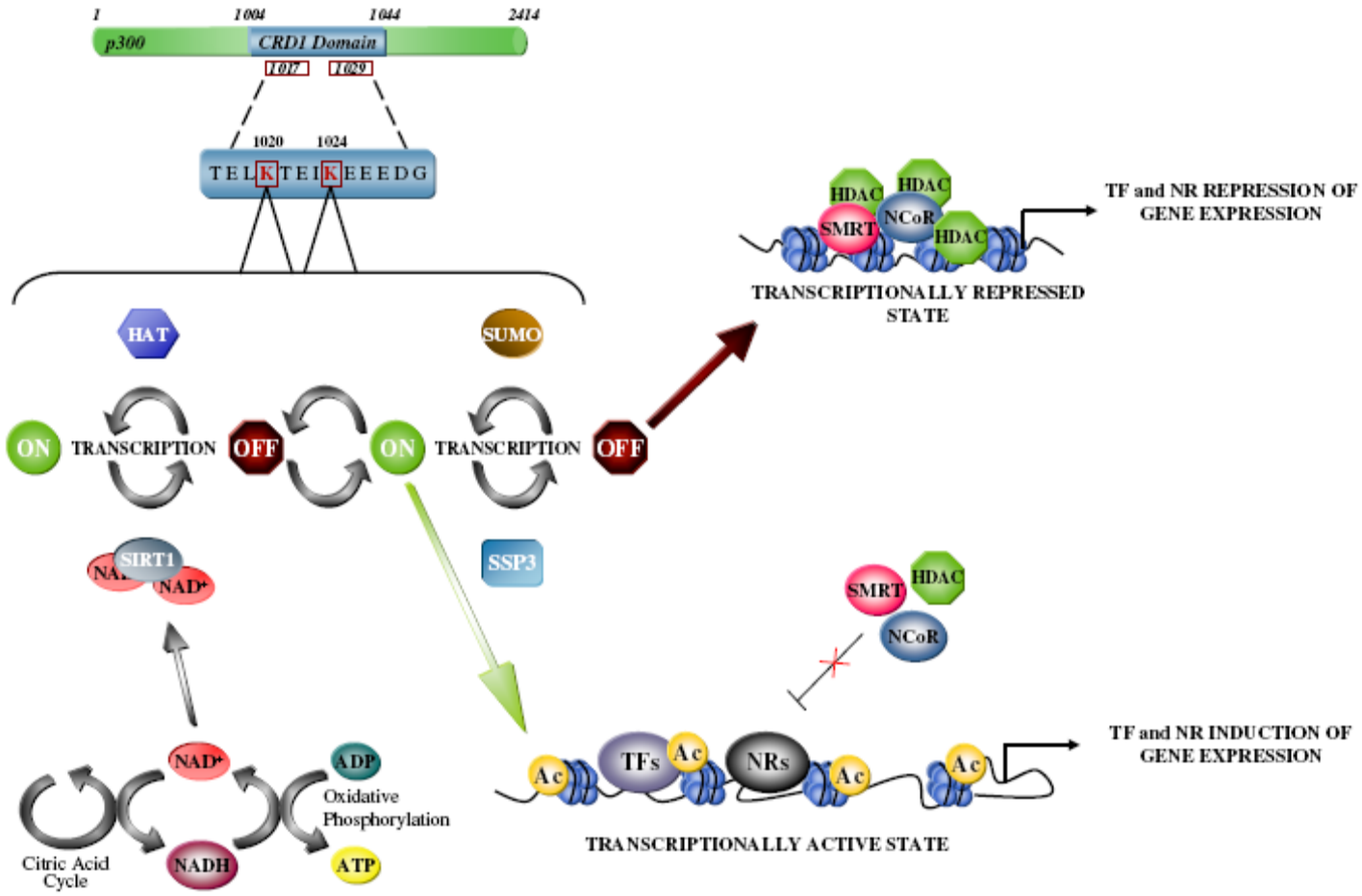
34. Puigserver, P., Adelmant, G., Wu, Z., Fan, M., Xu, J., O'Malley, B., and Spiegelman, B. M. Activation of PPARgamma coactivator-1 through transcription factor docking. Science, *286:* 1368-1371, 1999.
35. Yu, X., Guo, Z. S., Marcu, M. G., Neckers, L., Nguyen, D. M., Chen, G. A., and Schrump, D. S. Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. J Natl Cancer Inst, *94:* 504-513, 2002.
36. Kovacs, J. J., Murphy, P. J., Gaillard, S., Zhao, X., Wu, J. T., Nicchitta, C. V., Yoshida, M., Toft, D. O., Pratt, W. B., and Yao, T. P. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. Mol Cell, *18:* 601-607, 2005.
37. Mittal, R., Peak-Chew, S. Y., and McMahon, H. T. Acetylation of MEK2 and I kappa B kinase (IKK) activation loop residues by YopJ inhibits signaling. Proc Natl Acad Sci U S A, *103:* 18574-18579, 2006.
38. Schuur, E. R., Loktev, A. V., Sharma, M., Sun, Z., Roth, R. A., and Weigel, R. J. Ligand-dependent interaction of estrogen receptor-alpha with members of the forkhead transcription factor family. J Biol Chem, *276:* 33554-33560, 2001.
39. Yamamoto, H., Schoonjans, K., and Auwerx, J. Sirtuin functions in health and disease. Mol Endocrinol, *21:* 1745-1755, 2007.
40. Yang, T., Fu, M., Pestell, R., and Sauve, A. A. SIRT1 and endocrine signaling. Trends Endocrinol Metab, *17:* 186-191, 2006.
41. Bouras, T., Fu, M., Sauve, A. A., Wang, F., Quong, A. A., Perkins, N. D., Hay, R. T., Gu, W., and Pestell, R. G. SIRT1 deacetylation and repression of P300 involves lysine residues 1020/1024 within the cell-cycle regulatory domain 1. J Biol Chem, *280:* 10264-10276, 2005.
42. Gerhart-Hines, Z., Rodgers, J. T., Bare, O., Lerin, C., Kim, S. H., Mostoslavsky, R., Alt, F. W., Wu, Z., and Puigserver, P. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. Embo J, *26:* 1913-1923, 2007.
43. Rodgers, J. T., Lerin, C., Haas, W., Gygi, S. P., Spiegelman, B. M., and Puigserver, P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature, *434:* 113-118, 2005.
44. Nemoto, S., Fergusson, M. M., and Finkel, T. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1{alpha}. J Biol Chem, *280:* 16456-16460, 2005.
45. Fu, M., Liu, M., Sauve, A. A., Jiao, X., Zhang, X., Wu, X., Powell, M. J., Yang, T., Gu, W., Avantaggiati, M. L., Pattabiraman, N., Pestell, T. G., Wang, F., Quong, A. A., Wang, C., and Pestell, R. G. Hormonal control of androgen receptor function through SIRT1. MCB, *26:* 8122-8135, 2006.
46. Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., Leid, M., McBurney, M. W., and Guarente, L. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature, *429:* 771776, 2004.
47. Whittle, J. R., Powell, M. J., Popov, V. M., Shirley, L. A., Wang, C., and Pestell, R. G. Sirtuins, nuclear hormone receptor acetylation and transcriptional regulation. Trends Endocrinol Metab, 2007.
48. Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., Lavu, S., Wood, J. G., Zipkin, R. E., Chung, P., Kisielewski, A., Zhang, L. L., Scherer, B., and Sinclair, D. A. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature, *425:* 191-196, 2003.
49. Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F.,

Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P., and Auwerx, J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell, *127:* 1109-1122, 2006.
50. Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., Lakatta, E. G., Le Couteur, D., Shaw, R. J., Navas, P., Puigserver, P., Ingram, D. K., de Cabo, R., and Sinclair, D. A. Resveratrol improves health and survival of mice on a high-calorie diet. Nature, *444:* 337-342, 2006.
51. Ju, B. G., Lunyak, V. V., Perissi, V., Garcia-Bassets, I., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. Science, *312:* 1798-1802, 2006.
52. Mostoslavsky, R., Chua, K. F., Lombard, D. B., Pang, W. W., Fischer, M. R., Gellon, L., Liu, P., Mostoslavsky, G., Franco, S., Murphy, M. M., Mills, K. D., Patel, P., Hsu, J. T., Hong, A. L., Ford, E., Cheng, H. L., Kennedy, C., Nunez, N., Bronson, R., Frendewey, D., Auerbach, W., Valenzuela, D., Karow, M., Hottiger, M. O., Hursting, S., Barrett, J. C., Guarente, L., Mulligan, R., Demple, B., Yancopoulos, G. D., and Alt, F. W. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell, *124:* 315-329, 2006.
53. Lin, S. J., Defossez, P. A., and Guarente, L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science, *289:* 2126-2128, 2000.
54. Lin, S. J., Ford, E., Haigis, M., Liszt, G., and Guarente, L. Calorie restriction extends yeast life span by lowering the level of NADH. Genes Dev, *18:* 12-16, 2004.
55. Fulco, M., Schiltz, R. L., Iezzi, S., King, M. T., Zhao, P., Kashiwaya, Y., Hoffman, E., Veech, R. L., and Sartorelli, V. Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state. Mol Cell, *12:* 51-62, 2003.
56. Grinspoon, S., Corcoran, C., Lee, K., Burrows, B., Hubbard, J., Katznelson, L., Walsh, M., Guccione, A., Cannan, J., Heller, H., Basgoz, N., and Klibanski, A. Loss of lean body and muscle mass correlates with androgen levels in hypogonadal men with acquired immunodeficiency syndrome and wasting. J Clin Endocrinol Metab, *81:* 4051-4058, 1996.
57. Grool, L. J. D. Anabolic steroids. In (ed), Endocrinology, 3rd ed.*:* 2362-2369, 1995.
58. Altenberg, B. and Greulich, K. O. Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. Genomics, *84:* 1014-1020, 2004.
59. Chowdhury, S. K., Gemin, A., and Singh, G. High activity of mitochondrial glycerophosphate dehydrogenase and glycerophosphate-dependent ROS production in prostate cancer cell lines. Biochem Biophys Res Commun, *333:* 1139-1145, 2005.
60. Baron, A., Migita, T., Tang, D., and Loda, M. Fatty acid synthase: a metabolic oncogene in prostate cancer? J Cell Biochem, *91:* 47-53, 2004.
61. Rossi, S., Graner, E., Febbo, P., Weinstein, L., Bhattacharya, N., Onody, T., Bubley, G., Balk, S., and Loda, M. Fatty acid synthase expression defines distinct molecular signatures in prostate cancer. Mol Cancer Res, *1:* 707-715, 2003.

Figure Legends

Figure 1: The histone acetylatransferase (HAT) protein, p300, is capable of regulating gene expression through transcription factors (TFs) and nuclear receptors (NRs) binding to DNA specific sequences within euchromatin. This effect is governed by K1020 and K1024 acetylation within p300's CRD1 domain. SIRT1, the function of which is induced by NAD^+ produced as a byproduct of oxidative phosphorylation, serves as the master regulatory protein in this process.

Figure 1


1
1004
1044
2414
p300
CRD1 Domain
1017
1029
1020
1024
T E L K T E I K E E E D G
HAT
SUMO
ON
TRANSCRIPTION
OFF
ON
TRANSCRIPTION
OFF
SIRT1
NAD+
SSP3
HDAC
HDAC
SMRT
NCoR
HDAC
TRANSCRIPTIONALLY REPRESSED STATE
TF and NR REPRESSION OF GENE EXPRESSION
SMRT
HDAC
NCoR
NAD+
ADP
Oxidative Phosphorylation
NADH
ATP
Citric Acid Cycle
Ac
TFs
Ac
NRs
Ac
Ac
TRANSCRIPTIONALLY ACTIVE STATE
TF and NR INDUCTION OF GENE EXPRESSION