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Acetylation of Yeast AMPK Controls Intrinsic Aging Independently of Caloric Restriction

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SUMMARY

Acetylation of histone and nonhistone proteins is an important posttranslational modification affecting many cellular processes. Here, we report that NuA4 acetylation of Sip2, a regulatory β subunit of the Snf1 complex (yeast AMP-activated protein kinase), decreases as cells age. Sip2 acetylation, controlled by antagonizing NuA4 acetyltransferase and Rpd3 deacetylase, enhances interaction with Snf1, the catalytic subunit of Snf1 complex. Sip2-Snf1 interaction inhibits Snf1 activity, thus decreasing phosphorylation of a downstream target, Sch9 (homolog of Akt/S6K), and ultimately leading to slower growth but extended replicative life span. Sip2 acetylation mimetics are more resistant to oxidative stress. We further demonstrate that the anti-aging effect of Sip2 acetylation is independent of extrinsic nutrient availability and TORC1 activity. We propose a protein acetylation-phosphorylation cascade that regulates Sch9 activity, controls intrinsic aging, and extends replicative life span in yeast.

INTRODUCTION

Reversible acetylation and deacetylation of histones are important in regulating chromatin structure and controlling transcription of genes that are crucial for maintenance of cell viability (Lin et al., 2008). Among the histone deacetylases, Sir2 (yeast homolog of mammalian SIRT1; Donmez and Guarente, 2010) and Rpd3 (yeast homolog of mammalian HDAC1; Willis-Martinez et al., 2010) are especially important in life span regulation in yeast (Chang and Min, 2002; Jiang et al., 2002). Sir2 extends replicative life span partially via deacetylating histone H4 lysine 16 that compromises transcriptional silencing (Dang et al., 2009; Imai et al., 2000; Sinclair and Guarente, 1997). However, the life span discrepancies between substrate histone mutants and the acetyltransferase/deacetylase mutants (Dang et al., 2009) suggest possible roles of nonhistone acetylation substrates in mediating life span regulation by these (de)acetylating enzymes.

In a previous study, we identified many nonhistone substrates of the NuA4 complex, of which the catalytic subunit, Esa1, is the only essential histone acetyltransferase in yeast (Lin et al., 2009). We show here that NuA4 catalytic mutants have replicative life span defects caused by impaired acetylation of Sip2, a known replicative life span regulator (Ashrafi et al., 2000). Sip2 is one of three β regulatory subunits of the Snf1 complex and the only β subunit implicated in yeast replicative aging (Ashrafi et al., 2000; Lin et al., 2003). The Snf1 complex contains (1) a catalytic α subunit, Snf1, which is an AMP-activated serine/threonine protein kinase, (2) a γ subunit, Snf4, (3) and one of the three β regulatory subunits—Sip1, Sip2, or Gal83—each with a distinctive substrate specificity (Schmidt and McCartney, 2000). In addition to being required for transcription of glucose-repressed genes and utilization of carbon sources other than glucose (Amodeo et al., 2007), Snf1 is also a key player in the response to cellular stress (Sanz, 2003). Importantly, Snf1 activity increases in aged cells even when ambient glucose is abundant (Ashrafi et al., 2000; Hedbacker and Carlson, 2008). Null mutations in *SIP2*, the *SNF1* repressor, decrease life span; these can be rescued by deletion of *SNF4*, the *SNF1* activator (Guarente and Kenyon, 2000).

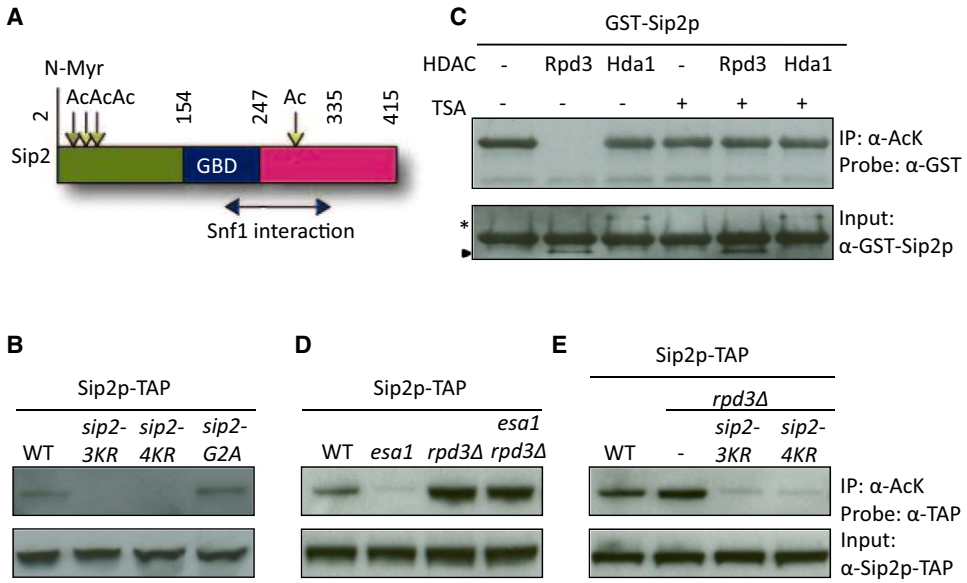

Figure 1. Sip2 Is Acetylated at Four Lysine Sites by Esa1, and Rpd3 Is the Counteracting Deacetylase

(A) Cartoon of Sip2 structural domains (adapted from Hedbacker and Carlson, 2008). Mass spectrometry identified four acetylated lysine residues of Sip2, K12, K16, K17, and K256. Numbers indicate amino acid residues. (Vertical straight line) Myristoylation site; (downward arrows) acetylation sites; (right-left arrow) regions mapped as sufficient for Snf1 interaction by deletion analysis (Amodeo et al., 2007). Ac, acetylation; N-Myr, N-myristoylation; GBD, glycogen-binding domain.

(B) Chromosomally integrated *sip2-3KR* and *sip2-4KR*, but not *sip2-G2A*, mutants are hypoacetylated in vivo. The *sip2-G2A* mutant blocks myristoylation. *sip2-3KR*, *sip2-K12/16/17R*; *sip2-4KR*, *sip2-K12/16/17/256R*.

(C) Rpd3, but not Hda1, removes Sip2 acetylation in vitro. Deacetylation reaction is inhibited by addition of trichostatin A (TSA). Asterisk indicates Hda1-TAP; arrowhead indicates Rpd3-TAP.

(D) Sip2 is hypoacetylated in strains carrying the Ts allele of *ESA1*, *esa1-531*, but is hyperacetylated in *rpd3Δ*; deletion of *RPD3* rescues the acetylation defect of *esa1-531*.

(E) Increased Sip2 acetylation in *rpd3Δ* is blocked when lysines are mutated to arginines.

See also Figure S1 and Figure S2.

Here, we report that Sip2 acetylation is controlled by the NuA4 and its counteracting deacetylase, Rpd3 (Chang and Pillus, 2009). We examined replicative life span in various *SIP2* acetylation mutants and found that Sip2 acetylation extends yeast life span; besides, constitutive Sip2 acetylation mimetics nearly totally rescue the life span-shortening phenotype of the NuA4 catalytic mutant, indicating the critical role of Sip2 acetylation in life span modulation. We further investigated glucose limitation, peroxide sensitivity, and age-associated changes in Sip2 acetylation, established the role of Sip2 acetylation in controlling Snf1 interactions and activities. Finally, we established that Sch9, the yeast homolog of Akt and S6K (Madia et al., 2009), was a common downstream target of two distinct replicative life span regulating pathways: the intrinsic aging defense pathway described here controlled by Snf1 kinase and the extrinsic nutrient-sensing pathway regulated by TORC1.

RESULTS

Sip2 Acetylation Is Controlled by NuA4 and Rpd3

To investigate the function of Sip2 acetylation, we identified four acetylated lysine residues (K12, K16, K17, and K256) (Figure 1A), using tandem mass spectrometry (Figures S1A–S1C available online). We then created unacetylable lysine-to-arginine mutant constructs at these four sites in various combinations by site-directed mutagenesis and introduced these mutant *SIP2* constructs into the endogenous chromosomal locus (Toulmay and Schneiter, 2006). Using a previously described reverse IP approach (Lin et al., 2009), we showed that Sip2 is hypoacetylated when the first three (K12, K16, and K17 [*3KR*]) or all four (K12, K16, K17, and K256 [*4KR*]) lysines are mutated (*Sip2-3KR* or *-4KR*; Figures S1D, Figure 1B). We also created a chromosomally integrated *SIP2* glycine 2-to-alanine mutant (*sip2-G2A*) to mimic the short-lived, non-N-myristoylated species of Sip2 (Ashrafi et al., 2000). We found that *sip2-G2A* did not have acetylation defects (Figure 1B). An in vitro deacetylation reaction carried out with purified Rpd3-TAP and Hda1-TAP (Figure S2A) revealed that Rpd3 treatment abolished the acetylation signal of Sip2 (Figure 1C). We demonstrated the activity of purified Hda1-TAP by showing that it deacetylated lysine 14 of FLAG-Htz1 purified from *hda1Δ* strain (Figure S2B) (Lin et al., 2008). As will be shown below, mutagenesis of the Sip2 acetylation sites affects replicative aging. The findings are consistent with a previous report that Rpd3, but not Hda1, is involved in replicative life span regulation (Kim et al., 1999). Previously we have shown that the acetylation signals of Sip2 are virtually completely dependent on NuA4/Esa1 both in vivo and in vitro (Lin et al., 2009). Importantly, simultaneous deletion