Human Sir2 and the ‘silencing’ of p53 activity

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Members of the evolutionarily conserved silent information regulator 2 (Sir2) protein family are nicotinamide adenine dinucleotide (NAD⁺)-dependent histone deacetylases. In yeast, the founding Sir2 protein is known to function in transcriptional silencing processes through the deacetylation of histones H3 and H4, thus setting up a repressive chromatin structure. Yeast and Caenorhabditis elegans Sir2 are also involved in regulating the life span of these organisms. Until recently, the function of mammalian Sir2 family members was completely unknown. However, several recent studies have now determined a remarkable function for the human SIRT1 protein, which is the closest human homolog of yeast Sir2. SIRT1 specifically associates with the p53 tumor suppressor protein and deacetylates it, resulting in negative regulation of p53-mediated transcriptional activation. Importantly, p53 deacetylation by SIRT1 also prevents cellular senescence and apoptosis induced by DNA damage and stress.

Post-translational histone modifications play a critical role in the regulation of eukaryotic gene expression. The highly conserved N-terminal tails of histones H3 and H4 contain several lysine residues that are acetylated by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs). In general, histone acetylation is associated with high levels of transcription and deacetylation is associated with transcriptional repression. This notion corresponds well with multiple examples of HATs that are found in coactivator complexes and HDACs that are components of corepressor complexes. However, it has become increasingly clear that non-histone proteins, including many transcription factors, are also acetylation targets for HATs [1]. So widespread is protein acetylation that it might eventually be found to rival phosphorylation in its importance within cellular signaling cascades. Recent exciting studies now implicate the silent information regulator 2 (Sir2) family of deacetylases as major players in this niche [2–5], thus extending the role of Sir2 beyond heterochromatic silencing.

The emergence of Sir2 as a histone deacetylase

The HDACs are divided into three classes based on sequence homology [6]. Class I enzymes have high homology to the yeast Rpd3 deacetylase and the class II enzymes are homologous to the yeast HDA1 deacetylase. The third class of HDACs are the Sir2 family of enzymes. Sir2 from the budding yeast Saccharomyces cerevisiae was the founding member of this highly conserved gene family, and was identified as a gene required for transcriptional silencing at the HM silent mating-type loci [7]. Related proteins have been identified in Archea, eubacteria, fungi, and metazoans, including humans [8]. The number of Sir2-like proteins in each species varies. For example, in addition to Sir2 itself, S. cerevisiae has four other family members named Hst1 through Hst4 [8]. Human cells contain eight different family members named SIRT1 through SIRT8 [9]. The Sir2 proteins were only recently shown to have histone deacetylase activity and their distinguishing feature is that they rely completely on the oxidized form of nicotinamide adenine dinucleotide (NAD⁺) for activity [10–12]. NAD⁺ is a crucial electron-accepting cofactor used in multiple oxidation reactions, including many related to energy utilization.

The critical role of yeast Sir2 in transcriptional silencing is well established so the fact that Sir2 is a histone deacetylase makes a lot of sense. Why, however, does Sir2 HDAC activity require NAD⁺ as a cofactor? It turns out that Sir2 hydrolyzes NAD⁺ as part of the deacetylase reaction, resulting in nicotinamide and O-acetyl-ADP-ribose products [7]. The acetyl group is removed from a lysine residue and transferred to the 2’ or 3’ hydroxyl group of the ribose ring of ADP ribose [13,14]. The hydrolysis of NAD⁺ is therefore coupled directly to the...
deacetylation reaction and for every acetyl group removed, one molecule of nicotinamide and one molecule of O-acetyl-ADP-ribose are produced [7]. It has been proposed that the O-acetyl-ADP-ribose might act as a novel signaling molecule [15]. In support of this hypothesis, it was recently shown that microinjected O-acetyl-ADP-ribose can block Xenopus oocyte maturation or embryonic cell division in blastomeres [16].

It is clear that not all of the Sir2 family members are involved in transcriptional silencing in the way that yeast Sir2 is. For example, there are no histones in bacteria and the Sir2 homolog in Salmonella, CobB, performs a phosphoribosyltransferase reaction in the synthesis of vitamin B12 [17]. Furthermore, only some of the Sir2-like proteins tested so far are localized in the nucleus. Three major questions in the Sir2 field, therefore, are: (1) What are the in vivo targets for yeast Sir2 and its multiple family members in other organisms? (2) How does deacetylation lead to transcriptional silencing? (3) What is the significance of an NAD+-dependent deacetylase carrying out these reactions?

**Non-histone targets for Sir2-like proteins**

In the original papers describing NAD+-dependent deacetylase activity in yeast and mammalian Sir2, lysine 16 on the N-terminal tail of histone H4 and lysines 9 and 14 on the N-terminal tail of histone H3 were shown to be deacetylation targets of Sir2 in vitro [10,11]. Recent chromatin immunoprecipitation experiments have shown that histones H3 and H4 associated with the silent rDNA and telomeric loci are deacetylated in a Sir2-dependent manner in vivo [18,19]. Therefore, histones are likely to be targets of yeast Sir2 in vivo. However, these data do not say definitively that Sir2 performed the deacetylation directly, and there is a good possibility that Sir2 will also be found to deacetylate other proteins in yeast.

For many years prior to the first identification of a HAT, it was known that many proteins are N-terminally acetylated, and that several non-histone chromatin proteins such as HMG1 are acetylated. With the identification of multiple HATs, there are now multiple non-histone transcription factors known to be acetylated in vitro and in vivo [1]. Perhaps the most prominent example is the p53 tumor suppressor protein, which is acetylated by the coactivator CBP/p300 on Lys382 and to a lesser extent on Lys373 and Lys381 [20]. p300/CBP-associated factor (PCAF) can also acetylate p53 on Lys320 in vitro. Acetylation of p53 by p300 stimulates its transcriptional activation activity through enhanced DNA binding, coactivator recruitment, and improved p53 stability [21]. Three recent studies demonstrated that the closest human and mouse homologs of yeast Sir2 (SIRT1 and mSIR2α, respectively) can specifically deacetylate Lys382 of p53 [3–5]. These reports come on the heels of an earlier report of a trichostatin A (TSA)-sensitive HDAC1-containing complex that deacetylates p53 [20]. TSA specifically inhibits HDAC1-like histone deacetylase activity, not Sir2 activity. Human SIRT1 can therefore act as a factor deacetylase (FDAC) and firmly establishes that Sir2 does not solely function to facilitate heterochromatin formation.

In another intriguing paper, mSIR2α was recently shown to deacetylate an RNA polymerase I (Pol I) transcription factor called TAF, 68 in vitro [2]. TAF, 68 is a component of the 11F-18/SIR 1 complex that binds to the rDNA promoter and initiates rRNA transcription through recruitment of Pol I [2]. TAF, 68 is acetylated by PCAF in vitro [2]. In this case TAF, 68 deacetylation by mSIR2α results in the repression of Pol I-mediated rDNA transcription. The rate of rDNA transcription is tightly regulated by cellular growth conditions, suggesting that mSIR2α could be a putative sensor of NAD± concentration that helps to maintain the appropriate level of Pol I transcription.

**Sir2 negatively regulates p53 activity**

The three recent papers mentioned earlier show that SIRT1 localizes in the nucleus, where it physically interacts with, and deacetylates, p53 [3–5]. Specifically, the Lys382 residue is deacetylated by SIRT1 in an NAD+-dependent, but TSA-insensitive, manner. Because an HDAC1 complex also deacetylates Lys382 of p53 [20], one can speculate that acetylation of Lys382 is very important and that SIRT1 and HDAC1 overlap in their p53 regulatory functions or that they deacetylate p53 in response to independent signals. Indeed, maximum p53 acetylation in response to the DNA-damaging drug etoposide was observed only when HDAC1 was inhibited with TSA and SIRT1 was inhibited by nicotinamide simultaneously [5].

Because p53 is a transcriptional activator, the downstream effect of p53 activation by acetylation is increased transcription of p53 target genes such as...
p21. This results in cell-cycle arrest, senescence, or the initiation of programmed cell death (apoptosis) (Fig. 1). Each of the three reports demonstrated that SIRT1-mediated deacetylation of Lys382 decreased p53-mediated transcriptional activation as measured by artificial reporter gene readouts or reduced endogenous p21 protein levels [3–5]. Importantly, expression of SIRT1 suppressed apoptosis induced by either DNA damage or oxidative stress (Fig. 1). SIRT1 expression also increased the survival of cells to ionizing radiation [4]. SIRT1 overexpression therefore seems to mediate the survival of cells during periods of severe stress through the inhibition of apoptosis. Langley et al. take this a step further and show that Sir2 is recruited to promyelocytic leukemia protein (PML) nuclear bodies upon overexpression of PML or oncogenic Ras (H-a-rasV12) [3]. These nuclear bodies contain p53, among other proteins, and have been implicated in transcriptional regulation and, interestingly, apoptosis.

These results are very exciting in light of the known role of yeast Sir2 in regulating cellular life span [22]. Deletion of Sir2 shortens yeast life span, whereas overexpression of Sir2 extends life span [22]. Furthermore, increased Sir2 dosage in Caenorhabditis elegans also extends life span [22]. C. elegans has a p53 homolog [23], but, as far as we know, yeast cells do not. It is still not known whether any of the mammalian Sir2-family members, including SIRT1, play a role in regulating cellular or organismal life span. One recent report has demonstrated that mice engineered to have high p53 activity have a short life span, even though they are resistant to tumor formation [24]. p53 might therefore have a more direct role in life span regulation than previously thought. It is possible that post-translational modifications of p53, including deacetylation by SIRT1, could play a critical role in modulating the proper level of p53 activity that strikes a balance between tumor suppression and manipulation of longevity.

Because SIRT1 is dependent on NAD+, as a cofactor, the metabolic state of the cell could potentially influence a critical cell fate decision during times of DNA damage and other cellular stresses (Fig. 1). If cellular NAD+ concentrations are low, then SIRT1 deacetylase activity could be attenuated, thus increasing the chances of a cell becoming senescent or apoptotic through the acetylated form of p53. In support of this idea, blocking the yeast NAD+-salvage pathway through deletion of the nicotinic acid phosphoribosyltransferase gene, NPT1, causes a threefold reduction in NAD+ concentration, and the mutant strains have transcriptional silencing defects [12,25].

**Concluding remarks**

How far removed is the new activity of SIRT1 from transcriptional silencing? Because SIRT1 associates physically with p53, this raises the possibility that a p53 complex containing SIRT1 might be targeted to p53-responsive promoters to deacetylate the surrounding histones and repress transcription. There is already precedence for Sir2 targeting to specific promoters in yeast. The Hst1 protein is highly similar to Sir2 and is targeted to meiotic gene promoters by the Sum1 transcriptional repressor [26]. It should also be noted that only a fraction of SIRT1 interacts with p53 and is localized in PML nuclear bodies [3,4]. It is therefore very unlikely that the only function of mammalian SIRT1 is deacetylation of p53. Given the large number of Sir2 family members, more fascinating functions for these NAD+-dependent deacetylases are sure to be identified.

**References**


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