SIRT1 Shows No Substrate Specificity in Vitro*

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SIR2 is a key regulator of the aging process in many model organisms. The human ortholog SIRT1 plays a pivotal role in the regulation of cellular differentiation, metabolism, cell cycle, and apoptosis. SIRT1 is an NAD+-dependent deacetylase, and its enzymatic activity may be regulated by cellular energy. There is a growing number of known SIRT1 substrates that contain ε-acetyl lysine but for which no obvious consensus sequence has been defined. In this study, we developed a novel unbiased method to identify deacetylase sequence specificity using oriented peptide libraries containing acetylated lysine. Following incubation with SIRT1, the subset of deacetylated peptides was selectively captured using a photocleavable N-hydroxysuccinimide (NHS)-biotin linker and streptavidin beads and analyzed using mass spectrometry and Edman degradation. These studies revealed that substrate recognition by SIRT1 does not depend on the amino acid sequence proximate to the acetylated lysine. This result brings us one step closer to understanding how SIRT1 and possibly other protein deacetylases chose their substrate.

SIR2 is a major regulator of the aging process. SIR2 functions as an NAD+-dependent deacetylase, which removes an acetyl group from the ε-amino group of lysine residues and is conserved from bacteria to higher eukaryotes (1) (for a review, see Ref. 2). This unique enzymatic reaction, which depends on NAD+ availability, may connect cellular energy with SIR2 activity. In Saccharomyces cerevisiae, SIR2 overexpression dramatically increases life span, whereas deletion of the SIR2 locus causes premature aging of the yeast mother cell (3). In worms, SIR2 overexpression extends life span (4).

In humans, there are seven SIR2 homologs (SIRT1 to -7); of them, SIRT1 is the SIR2 ortholog (2, 5). SIRT1 negatively regulates the differentiation of muscle (6) and fat cells (7) and may be a major contributor of caloric restriction in mammals (7). A variety of SIRT1 substrates have been discovered. These substrates include histones (1, 8), the p53 suppressor gene (9, 10), the FOXO transcription factors (11, 12), TAFI68 (13), and Ku70 (14). These substrates indicate that SIRT1 might be involved in apoptosis, cell cycle regulation, transcription, and many other cellular and organismal regulatory pathways. Indeed, SIRT1-deficient mice have a severe phenotype such as small size, delay in eyelid opening, cardiac defect, and sterility (15, 16).

The structure of the SIRT1 homolog Af2 has been determined both in the presence and absence of a bound acetylated p53-derived peptide (17, 18). Two amino acids at the N terminus from the ε-Ac-lysine and five amino acids C-terminal to the Ac-lysine of p53 peptide were well visualized in the electron density map, which suggests that they may play a role in the enzyme-substrate interaction (18). However, the structure did not improve our understanding of whether the amino acids flanking the ε-Ac-lysine residue are important for SIRT1 substrate recognition.

No known SIRT1 substrates have been identified using an unbiased substrate search method, and the SIRT1 deacetylation consensus sequence, if one exists, has not been identified to date. In an attempt to determine an optimal deacetylation motif for SIRT1, we developed a novel deacetylase capture assay using unbiased oriented peptide libraries. Peptide library screening has proven to be a powerful method for mapping the consensus motifs recognized and phosphorylated by protein kinases or bound by modular peptide binding domains (19). For example, a variety of substrates for protein kinase C (20), AKT/protein kinase B (21), and ATM (22) were discovered using this method. In this paper, we describe the use of an acetyl-lysine-based oriented peptide approach combined with biotin-avidin capture technology to query SIRT1 deacetylated peptides for a common recognition motif. Using this method, we discovered that SIRT1 selection for substrate peptides does not depend on the amino acids proximal to ε-Ac-lysine and verified these findings by detailed enzymatic kinetic analysis.

MATERIALS AND METHODS

Purification of the SIRT1 Protein—293T cells were transfected with SIRT1-FLAG plasmid (7) by JetPEI (Qiogene, Carlsbad, CA). Cells were lysed 48 h post-transfection in Nonidet P-40 extraction buffer (50 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 1% Nonidet P-40) in the presence of protease inhibitor mixture—EDTA (Complete; Roche Applied Science). FLAG-tagged proteins were immunoprecipitated with anti-FLAG M2-agarose affinity gel (Sigma) for 2 h at 4 °C. Immunoprecipitated material was washed three times in the extraction buffer. FLAG-SIRT1 was eluted by incubation with FLAG peptide (Sigma) and dialyzed against phosphate-buffered saline.

Peptide Library Experiment—200 µg of the peptide library or the SIRTide (Tufts Medical School, Boston, MA) was incubated overnight at 37 °C in 50 mM phosphate buffer (pH 8.0), 4 mM MgCl2, 0.5 mM NAD, 2 mM dithiothreitol in the presence or absence of 1 µg of purified mammalian SIRT1. Next, 3 µl of 25 µM of photocleavable biotin derivative (photocleavable (PC)-biotin-NHS) (23) (AmberGen, Boston, MA), 80 µl of dimethylformamide, and 80 µl of 1 M NaHCO3 were added and incubated for 1 h at room temperature. To this solution, 500 µl of NeutAvidin-agarose (Pierce) was added and incubated at room temperature for 30 min. Beads were washed with 1 ml of 50 mM HEPES (pH 7.0) (three times), 50 mM NH4OAc (pH 7.2) (three times), and 10% ethyl alcohol. Samples were eluted in 1 ml of 10% ethyl alcohol for 15 min by UV exposure (Blak Ray XX-15 UV lamp; Ultraviolet Products, San...
Gabriel, CA) at a distance of 15 cm (emission peak 365 nm, lamp intensity 1.1 milliwatt/cm² at a distance of 31 cm). The samples were dried, resuspended in 40 μl of 7.5% ammonia in H₂O, and incubated overnight to remove the Fmoc. Samples were dried, resuspended in 10 μl of H₂O, and analyzed by MALDI-TOF and Edman degradation (Tufts Medical School, Boston, MA).

Library Synthesis—A peptide library containing the sequences Fmoc-MA XXXX-AcK-XXXXX AEEE, where X indicates all amino acids except Lys or Cys, was synthesized using solid phase peptide synthesis with N-(9-fluorenyl)methoxycarbonyl-protected amino acids and standard BOP/HOBt coupling chemistry (Tufts Medical School, Boston, MA). Individual peptides were synthesized in a similar manner.

Mass Spectrometry—Mass spectrometry was performed with the Voyager DE-TOF mass spectrometer (PerkinElmer Life Sciences) with delayed ion extraction and nitrogen laser (337 nm) and analyzed by GRAMS software. Samples were desalted prior to analysis using ZipTip C18 pipette tips (size P10) (Millipore Corp.) according to the manufacturer's instructions, and then 1 μl of sample solution was deposited on a steel target plate followed by 1 μl of matrix solution, air-dried, and spotted. α-Cyano-4-hydroxycinnamic acid (Aldrich) at a concentration of 10 mg/ml (5:4:1, v/v/v, acetonitrile/water/3% trifluoroacetic acid) was used as a matrix.

Kinetic Analysis—Briefly, synthesized acetylated peptides were resuspended in phosphate buffer and stored frozen at −70 °C. Peptide concentration was determined by amino acid analysis (Dana-Farber Cancer Institute, Boston, MA). Deacetylase reactions were carried out in a total volume of 16 μl of assay buffer containing 65 ng of bacterial expressed SIRT1, 150 μM NAD (N-1511; Sigma), 20 μM [carbonyl-14C]NAD (55 mCi/mmol of CFA372; Amersham Biosciences), and serially diluted peptide series (from 0.125 to 125 μM). The reaction was initiated with the addition of enzyme to the substrate mixture. The mixture was incubated for 50 min at 37 °C in a 96-well V-bottom polypropylene plate (catalog no. 615201; Greiner). AA assay buffer

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The abbreviations used are: Fmoc, N-(9-fluorenyl)methoxycarbonyl; MALDI, matrix-assisted laser desorption-ionization; TOF, time-of-flight; NHS, N-hydroxysuccinimide; PC, photocleavable; LC, linker-coupled; PML, promyelocytic leukemia.

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**FIG. 1.** A peptide library approach for capture and analysis of SIRT1 deacetylation products. A, the degenerate peptide library was incubated with SIRT1 for various periods of time and the subset of SIRT1-deacetylated peptides was purified away from the acetylated starting material by incubation with NHS-PC-LC-biotin. Because the amino terminus has been blocked with an Fmoc group, the NHS group reacts specifically with the free ε-amino group of the central lysine residue in the library, which is present only in the deacetylated population. B, the biotinylated deacetylated peptides were captured on streptavidin-agarose beads, followed by extensive washing to remove nonbiotinylated (acyetylated) peptides. C, the beads were irradiated with 365-nm UV light to reverse the NHS cross-link and liberate the deacetylated peptide mixture. D, the released peptides were incubated with concentrated ammonia to cleave the N-terminal Fmoc protecting group and sequenced in bulk by Edman degradation.
No Substrate Specificity for SIRT1

RESULTS

To determine the requirements for substrate specificity for SIRT1 and ask whether there is a consensus sequence for its targets, we developed a new acetyl-lysine-oriented peptide library-based strategy for mapping SIRT1 substrate specificity (Fig. 1). A degenerate peptide library corresponding to the sequence Fmoc-MAXXXX-AcK-XXXXXAEVE was synthesized, where AcK indicates acetyl-lysine and X indicates all amino acids except Lys or Cys. Lys was omitted from the degenerate positions, and the N-terminus was blocked by Fmoc to ensure that the starting library contained no free amino groups in the absence of SIRT1-driven deacetylation. Cys was omitted to avoid problems with sequencing and oxidation.

The degenerate peptide library was incubated with SIRT1 for various periods of time, and the subset of SIRT1-deacetylated peptides was purified away from the acetylated starting material by incubation with (NHS-PC-linker-coupled (LC)-biotin). The NHS group reacts specifically with the free ε-amino group of the central lysine residue in the library, which is present only in the deacetylated population (23). The biotinylated, deacetylated peptides were captured on streptavidin-agarose beads, washed extensively to remove nonbiotinylated (acetylated) peptides. The beads were then irradiated with 365-nm UV light to reverse the NHS cross-link and liberate the deacetylated peptide mixture. The released peptides were incubated with concentrated ammonia to cleave the N-terminal Fmoc protecting group and sequenced in bulk by Edman degradation (Fig. 1).

The Met-Ala sequence was designed at the amino terminus of the peptide library to verify that any biotin-streptavidin-captured peptide obtained with this approach came from the substrate library mixture and to help quantify the amount of peptide present. The fixed Ala residue at position 13 in the library provides an estimate of how much peptide loss occurs during sequencing, whereas the poly(Glu) C-terminal tail prevents peptide washout during Edman degradation sequencing.

The strategy was initially optimized using an individual SIRT1 peptide Fmoc-MASTGG-AcK-DSTEMAEEE; SIRTide) in which the N-terminal half was derived from the histone H3 peptide amino acid sequence, a known SIRT1 substrate (1), and the C-terminal was derived from the RCH1 peptide sequence, a SIRT1 substrate that we recently identified (data not shown). This peptide included the same total number of amino acids and the same constant amino acid positions as the degenerate peptide library mixture and to help quantify the amount of the peptide library to verify that any biotin-streptavidin-captured peptide obtained with this approach came from the substrate library mixture and to help quantify the amount of peptide present. The fixed Ala residue at position 13 in the library provides an estimate of how much peptide loss occurs during sequencing, whereas the poly(Glu) C-terminal tail prevents peptide washout during Edman degradation sequencing.

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This peptide library/deacetylation/capture approach was capable of isolating peptides that were deacetylated by SIRT1 as evidenced by both mass spectrometry and Edman degradation.

To investigate the optimal SIRT1 deacetylation motif, the peptide library was incubated with SIRT1 for 15 h at 37 °C. Approximately 2% of the total peptide mixture was captured and photoreleased from the streptavidin-agarose beads (data not shown). These purified, deacetylated products were then sequenced by Edman degradation. The amount (mol %) of each amino acid in each of the degenerate positions was compared with its amount (mol %) in the starting library mixture in order to determine whether particular amino acids are selected at specific positions flanking the central acetyl-lysine residue. As shown by the resulting selectivity values in Table I (top), the relative abundance of the amino acids in each of the 9 degenerate positions flanking the fixed deacetylated Lys residue appears to be relatively invariant from cycle to cycle. Furthermore, each amino acid shows a similar overall abundance in the deacetylated peptide products to that present in the starting library mixture, as revealed by selectivity ratios close to 1.0. These data strongly argue that SIRT1 does not show any evidence for peptide motif specificity for SIRT1-medi-}

**Table I**

Lack of substrate specificity for SIRT1 using oriented peptide library screening

| Position | Asp | Asn | Ser | Glu | His | Ala | Arg | Tyr | Pro | Met | Val | Phe | Ile | Leu |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| -4       | 0.8 | 1   | 1.2 | 0.3 | 1   | 0.9 | 1.1 | 1.5 | 1.2 | 0.9 | 1.2 | 1   | 1   | 0.9 |
| -3       | 0.8 | 1   | 1.1 | 0.4 | 0.8 | 1   | 1.2 | 1.2 | 1.3 | 0.9 | 1.2 | 1.1 | 0.9 | 0.9 |
| -2       | 0.8 | 1   | 1.1 | 0.4 | 1   | 1   | 1.1 | 1.3 | 1.2 | 0.8 | 1.2 | 1   | 1.1 | 0.9 |
| -1       | 0.7 | 0.9 | 1.2 | 0.5 | 1   | 0.9 | 1.2 | 1.2 | 0.9 | 1.2 | 1.1 | 1   | 1   | 1   |
| Ac-K     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

| Position | Asp | Asn | Ser | Glu | His | Ala | Arg | Tyr | Pro | Met | Val | Phe | Ile | Leu |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| -4       | 0.9 | 0.6 | 1.1 | 0.9 | 0.6 | 0.8 | 1.3 | 0.9 | 0.6 | 0.9 | 1.3 | 1.3 | 1.7 | 1.2 |
| -3       | 0.9 | 0.6 | 0.7 | 0.5 | 0.4 | 0.9 | 1.1 | 0.9 | 0.5 | 1   | 1.5 | 1.6 | 2   | 1.4 |
| -2       | 0.8 | 0.9 | 0.8 | 0.8 | 0.4 | 0.8 | 1   | 1   | 0.5 | 1   | 1.4 | 1.5 | 1.8 | 1.4 |
| -1       | 0.5 | 0.6 | 0.8 | 0.9 | 0.4 | 0.9 | 1.1 | 1.1 | 0.6 | 1   | 1.4 | 1.8 | 1.8 | 1.4 |
| Ac-K     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

| Position | Asp | Asn | Ser | Glu | His | Ala | Arg | Tyr | Pro | Met | Val | Phe | Ile | Leu |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| -4       | 0.9 | 0.8 | 1.4 | 1   | 0.6 | 0.8 | 1.4 | 0.9 | 0.7 | 0.8 | 1.3 | 0.1 | 1.8 | 1.3 |
| -3       | 0.9 | 0.8 | 1   | 0.7 | 0.5 | 1   | 1   | 1.1 | 0.8 | 1   | 1.6 | 0.1 | 2.1 | 1.3 |
| -2       | 0.8 | 1.1 | 0.9 | 1   | 0.5 | 0.9 | 1   | 1.1 | 0.7 | 1   | 1.5 | 0.1 | 2.0 | 1.3 |
| -1       | 0.6 | 0.8 | 1   | 1   | 0.5 | 0.9 | 1.2 | 1.1 | 1.4 | 1.5 | 0.1 | 1.9 | 1.4 | 1.4 |
| Ac-K     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

| Position | Asp | Asn | Ser | Glu | His | Ala | Arg | Tyr | Pro | Met | Val | Phe | Ile | Leu |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| -4       | 0.8 | 1.2 | 1.1 | 1   | 0.5 | 1   | 1.1 | 0.9 | 1.2 | 0.9 | 1.2 | 1   | 1.1 | 1.5 |
| -3       | 0.7 | 1.2 | 1.1 | 1   | 0.5 | 1   | 1.1 | 1.1 | 1.4 | 0.9 | 1.2 | 0.1 | 1.5 | 1.3 |
| -2       | 0.6 | 1.3 | 1.2 | 1   | 0.5 | 1   | 0.9 | 1.1 | 1.1 | 0.9 | 1.2 | 0.1 | 1.4 | 1.4 |
| -1       | 0.8 | 1.2 | 1.2 | 1   | 0.5 | 1   | 1.1 | 0.9 | 1.1 | 1.1 | 0.9 | 1.2 | 0.1 | 1.4 |
| Ac-K     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

| Position | Asp | Asn | Ser | Glu | His | Ala | Arg | Tyr | Pro | Met | Val | Phe | Ile | Leu |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| -4       | 0.8 | 1.5 | 1.3 | 1   | 0.4 | 0.9 | 0.9 | 1   | 1.2 | 0.8 | 1.1 | 0.1 | 1.3 | 1.6 |
| -3       | 0.7 | 1.5 | 1.3 | 1   | 0.4 | 0.9 | 0.9 | 1   | 1.2 | 0.8 | 1.1 | 0.1 | 1.4 | 1.6 |
| -2       | 0.6 | 1.5 | 1.3 | 1   | 0.4 | 0.9 | 0.9 | 1   | 1.2 | 0.8 | 1.1 | 0.1 | 1.4 | 1.6 |
| -1       | 0.5 | 1.5 | 1.3 | 1   | 0.4 | 0.9 | 0.9 | 1   | 1.2 | 0.8 | 1.1 | 0.1 | 1.4 | 1.6 |
analysis of SIRT1 deacetylation was performed using wild-type and mutant peptides corresponding to known SIRT1 substrates p53 (p53tide) and H3/RCH1 (SIRtide). The deacetylation kinetics are shown in Fig. 4. Despite the striking sequence differences, the measured $K_m$ values were remarkably similar, with values of 61 $\mu M$ for p53tide and 50 $\mu M$ for SIRtide. Similarly, modification of the SIRtide peptide, in which three amino acids on each side of the acetylated Lys were replaced by Ala (SIRtide-ALa6) or the four C-terminal residues of SIRtide were replaced by those from a histone H3 peptide, had little effect on the resulting kinetic parameters. The $K_m$ values for all three substrates were not significantly different, whereas the SIRtide $V_{max}$ was 2-fold lower than the SIRtide-ALa6 and H3-like $V_{max}$. In conclusion, by using enzymatic assays, we verified the unexpected peptide library results showing that SIRT1 lacks strong sequence specificity for the amino acid residues proximal to the acetylated Lys.

**DISCUSSION**

In this work, we investigated the requirements for SIRT1 substrate sequence specificity using two approaches. First, we developed a novel peptide library-based screen to evaluate deacetylation substrate specificity in an unbiased manner, using chemical modification of free ε-amino groups of lysine, followed by biotin-streptavidin affinity capture, photorelease, and sequencing. The results of those experiments clearly showed that there was no preference for specific amino acids within 4 positions N-terminal and 5 positions C-terminal to the acetyl-lysine residue within peptide substrates. In contrast to the lack of sequence specificity we obtained for the SIRT1 protein deacetylation, all known protein kinases and phosphopeptide-binding domains show explicit sequence specificity in the region immediately surrounding the phosphorylated residue, and these sequence motifs are easily deduced using peptide library-based screening approaches (19–22).

Second, we determined kinetic parameters of SIRT1 deacetylation using wild-type and mutant peptides corresponding to the acetylation sites on several known in vivo SIRT1 substrates. These measurements revealed very similar $K_m$ and $V_{max}$ values for acetylated peptides with widely divergent sequences flanking the acetyl-lysine site, again suggesting a lack of sequence specificity. It is not known whether the lack of sequence specificity that we observed for SIRT1 is a general feature of all protein deactylases. The peptide library approach that we devised in this study, however, should allow us to investigate in future studies whether consensus recognition sequences exist for other deacetylase enzymes, such as the class I and class II protein deactylases (24). In addition, a variation of the amine modification/capture/photocleavage approach described here could be used to investigate the substrate specificity for protein acetyltransferases.

Given the relative lack of motif specificity of SIRT1 that we observed on peptide substrates, what factors might account for the substrate specificity of SIRT1 in vivo? One possibility is that bona fide in vivo substrates of SIRT1 contain the acetylated sequence within a defined tertiary fold in the substrate that is lacking in short unstructured peptide substrates. The x-ray structure of the SIRT1 homolog Af2 bound to an acetylated p53 peptide, however, argues against this. In that structure, tight binding of the deacetylase to a small peptide substrate was observed (18). This binding was mediated by insertion of the acetyl lysine residue into a hydrophobic tunnel and a series of hydrogen bonds between backbone atoms of two β-strands in Af2 to backbone atoms in the p53 peptide to form a staggered antiparallel three-stranded β-sheet (18). Interestingly, in this structure, the peptide side chains bracketing the acetyl-lysine interact with Af2 primarily through weak van der Waals interactions and two water-mediated hydrogen bonds. The lack of direct strong side chain interactions observed in the p53 peptide:Af2 structure is in good agreement with the lack of strong sequence specificity that we observed in our peptide substrate screening experiments.

An alternative explanation is that in vivo substrate specificity for SIR2 involves protein-protein interactions that occur outside of the SIRT1 active site. These interactions could be mediated by additional sites on SIRT1 itself or by other proteins present within larger SIRT1-containing complexes. In agreement with this, SIRT1 has been shown to interact with and localize to PML nuclear bodies (25), a well studied component of a subgroup of nucleoplasmic foci (26). Indeed, p53 is also recruited to PML nuclear bodies (25), a well studied component of a subgroup of nucleoplasmic foci (26). Indeed, p53 is also recruited to PML nuclear bodies (25), a well studied component of a subgroup of nucleoplasmic foci (26). Indeed, p53 is also recruited to PML nuclear bodies (25), a well studied component of a subgroup of nucleoplasmic foci (26). Indeed, p53 is also recruited to PML nuclear bodies (25), a well studied component of a subgroup of nucleoplasmic foci (26). Indeed, p53 is also recruited to PML nuclear bodies (25), a well studied component of a subgroup of nucleoplasmic foci (26). Indeed, p53 is also recruited to PML nuclear bodies (25), a well studied component of a subgroup of nucleoplasmic foci (26). Indeed, p53 is also recruited to PML nuclear bodies (25), a well studied component of a subgroup of nucleoplasmic foci (26). Indeed, p53 is also recruited to PML nuclear bodies (25), a well studied component of a subgroup of nucleoplasmic foci (26).

![Image](https://example.com/image.png)

**Fig. 4. Deacetylation kinetics of SIRT1 for peptide substrates.** Assays were performed as described under “Materials and Methods” using peptides corresponding to acetyl-lysine p53tide (A) and SIRtide (B).

**Table II**

| Sequence       | $K_m$ (μM) | $V_{max}$ (μM) | $K_m/V_{max}$ |
|----------------|------------|----------------|---------------|
| SIRtide        | 59 ± 6     | 610 ± 20       | 0.096         |
| SIRtide-ALa6   | 57 ± 9     | 1310 ± 70      | 0.043         |
| H3-like        | 87 ± 25    | 1150 ± 130     | 0.075         |

Amino acids are shown in single letter code.
to regulate transcription factors, such as peroxisome proliferator-
activated receptor γ, although it is not yet known whether NcoR
is a SIRT1 substrate (7, 29). Interestingly, the Werner syndrome
protein (WRN), mutations of which result in premature aging,
was recently shown to be an acetylated protein that localizes to
PML bodies (30). Given this co-localization and the known pivotal
roles of both SIR2 and WRN in life span determination (3, 31), it
is tempting to speculate that SIRT1-mediated deacetylation
of WRN might occur in PML bodies as part of the regulation
of aging. In addition to deactylases such as SIRT1, a variety
of acetyl transferases were recently shown to also be present
within PML bodies (11, 27), suggesting that dynamic acetyl-
ation and deacetylation events relevant to the control of
aging are occurring within these subcellular structures. In
this context, an improved understanding of acetylation and
deacetylation substrate determinants should facilitate the
elucidation of SIRT1 biology, caloric restriction, and aging
physiology.

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