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Sirtuins in Epigenetic Silencing and Control of Gene Expression in Model and Pathogenic Fungi

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Abstract

Fungi, including yeasts, molds, and mushrooms, proliferate on decaying matter and then adopt quiescent forms once nutrients are depleted. This review explores how fungi use sirtuin deacetylases to sense and respond appropriately to changing nutrients. Because sirtuins are NAD^+-dependent deacetylases, their activity is sensitive to intracellular NAD^+ availability. This allows them to transmit information about a cell's metabolic state on to the biological processes they influence. Fungal sirtuins are primarily known to deacetylate histones, repressing transcription and modulating genome stability. Their target genes include those involved in NAD^+ homeostasis, metabolism, sporulation, secondary metabolite production, and virulence traits of pathogenic fungi. By targeting different genes over evolutionary time, sirtuins serve as rewiring points that allow organisms to evolve novel responses to low NAD^+ stress by bringing relevant biological processes under the control of sirtuins.

Contents

INTRODUCTION

The fungal kingdom includes unicellular yeasts, molds, and mushrooms. These organisms are among the major decomposers in many ecosystems and can also parasitize animals and plants. As such, fungi experience cycles of boom, when nutrients are plentiful, and bust, once those nutrients are depleted. Consequently, fungi must sense and respond to nutrient availability to survive lean times.

One regulatory mechanism sensitive to nutrient availability is deacetylation by sirtuins. This family of deacetylases is notable because NAD^+ is required for catalytic activity. Consequently, the biological processes regulated by sirtuin deacetylation are responsive to intracellular NAD^+ levels and hence a cell's metabolic state. In this review, we explore how sirtuins are used by fungi to modulate their responses to low nutrient availability and more generally to regulate gene expression and genome stability. We take an evolutionary approach, comparing the functions of orthologous proteins in multiple species when possible. The relationships of the species discussed are presented in **Figure 1**.

Sirtuin: a member of a family of NAD^+-dependent deacetylases; named after *S. cerevisiae* Sir2

NAD^+ (nicotinamide adenine dinucleotide): a coenzyme required by many redox enzymes and by sirtuins

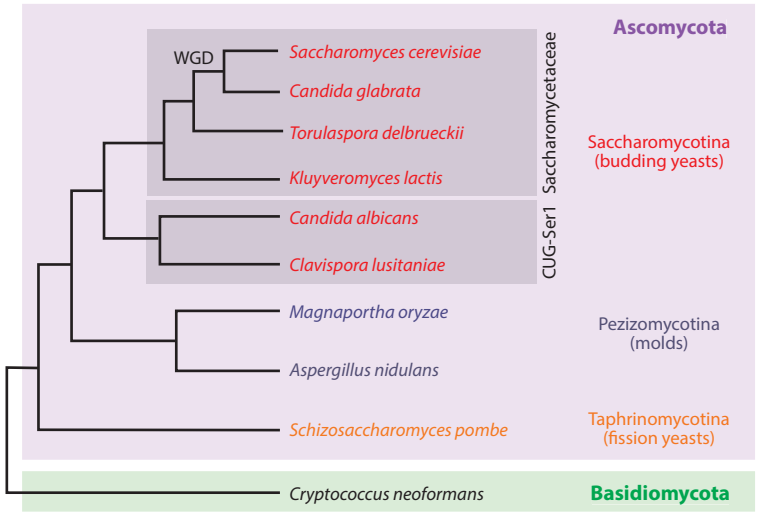

Figure 1

Relationships of species discussed in this review. Cladogram is not to scale. Abbreviation: WGD, whole-genome duplication.

REACTIONS CATALYZED BY SIRTUINS

Most sirtuins are deacetylases (59, 80), although some catalyze related deacylation reactions such as demalonylation and desuccinylation (30). Each deacetylation cycle (**Figure 2**) consumes one NAD^+ molecule. Nicotinamide is released, and the remaining ADP-ribose is joined to the acetyl group to generate *O*-acetyl-ADP ribose (79, 125). Consequently, NAD^+ is a required cofactor for deacetylation. In contrast, its reduced form, NADH, does not promote catalysis (59). The consumption of NAD^+ by sirtuins has long intrigued researchers because it is not energetically necessary—other families of deacetylases do not consume high-energy molecules. Therefore, it is widely thought that the requirement for NAD^+ enables sirtuins to sense and respond to fluctuations in intracellular NAD^+ levels.

Nicotinamide: one of the two bases in NAD^+ and a form of vitamin B_3; nicotinamide inhibits deacetylation by sirtuins

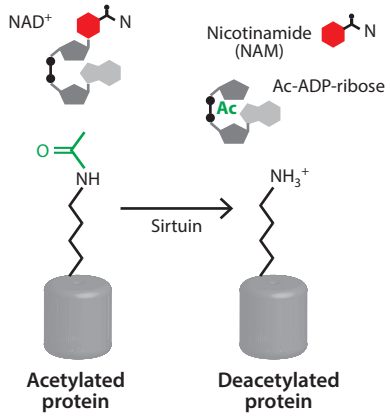

Figure 2

Deacetylation by sirtuins consumes NAD^+ and releases nicotinamide (NAM). Abbreviation: Ac, acetyl.

HISTONES AND NUCLEOSOMES

In eukaryotes, genomic DNA is packaged into nucleosomes, each consisting of ~150 base pairs of DNA wrapped around a core of eight histone proteins. Each nucleosome has two molecules of each of the four core histones: H2A, H2B, H3, and H4. The sirtuins discussed here primarily deacetylate lysines on the N-terminal tails of histones H3 and H4, resulting in condensed chromatin and lower transcription. In addition, Hst3 and Hst4 deacetylate Lys-56 of histone H3 (H3K56), which stabilizes the nucleosome.

Sirtuins deacetylate lysine side chains within proteins. Lysine acetylation can alter a protein's function by influencing its conformation or interactions. This occurs because an acetylated lysine is bulkier and has a neutral charge, whereas an unacetylated lysine is positively charged at physiological pH (**Figure 2**). Lysine acetylation can also compete with other modifications of lysines, including methylation and ubiquitination. The first acetylated proteins studied were histones. In general, acetylated histones are found in open chromatin that is permissive to transcription (euchromatin), whereas unacetylated histones are found in condensed chromatin that inhibits transcription (heterochromatin). Thus, deacetylation of histones by sirtuins represses transcription (see the sidebar titled Histones and Nucleosomes). In addition to histones, other proteins are also acetylated. Some, including transcription factors and metabolic enzymes, are deacetylated by sirtuins (36, 87).

NAD^+ HOMEOSTASIS IN FUNGI

Lysine acetylation: addition of an acetyl group (CH_3CO) to the terminal nitrogen of a lysine within a protein

Heterochromatin: condensed chromatin in which histones are hypoacetylated and transcription is blocked; also called silenced chromatin

Salvage pathway: A pathway that recycles nicotinamide and nicotinic acid to generate NAD^+

Niacin: a collective term for precursors of NAD^+, including nicotinic acid, nicotinamide, and nicotinamide riboside

NAD^+ is not only required for the sirtuin deacetylation reaction. It is also an essential cofactor for many enzymes, including a fourth of oxidoreductases (113). For many of these enzymes, the nicotinamide base of NAD^+ is a redox carrier, converting between its oxidized (NAD^+) and reduced (NADH) forms. Moreover, in metabolic pathways, NADH carries reducing equivalents. In particular, the breakdown of sugars, fatty acids, and other compounds reduces NAD^+ to NADH. This NADH is then oxidized back to NAD^+ by the electron transport chain, generating an electrochemical gradient to produce ATP. Yet another role of NAD^+ is to serve as the precursor of $NADP^+$, a redox carrier involved in biosynthetic reactions. Thus, NAD^+ is a critical metabolite that must be maintained in sufficient quantities. Moreover, NAD^+ levels could be affected by abrupt shifts in metabolic flux.

NAD^+ is a dinucleotide with two bases—nicotinamide and adenine. To generate NAD^+, nicotinamide can be synthesized de novo from tryptophan (kynurenine pathway), or it can be extracted from the environment through salvage pathways (**Figure 3**). In many fungi, synthesis is carried out by the *BNA* genes. However, some yeast species lack these genes and rely exclusively on salvage pathways. In these pathways, high-affinity transporters scavenge nicotinamide and related compounds (nicotinic acid and nicotinamide riboside) from the environment. These compounds are collectively called niacin or vitamin B_3. In *Saccharomyces cerevisiae*, NAD^+ levels are higher when niacin is available than when cells rely solely on de novo synthesis (12), indicating the importance of salvage in setting NAD^+ levels.

The intracellular NAD^+ concentration is determined by a dynamic network of metabolic pathways. NAD^+ production is offset by its consumption by enzymes including sirtuins, ADP-ribosyltransferases, and hydrolases. NAD^+ is also depleted by its reduction to NADH and its conversion to $NADP^+$. Although the mechanisms that regulate NAD^+ levels are incompletely understood, sirtuins contribute to this process. Some participate directly in feedback loops that induce

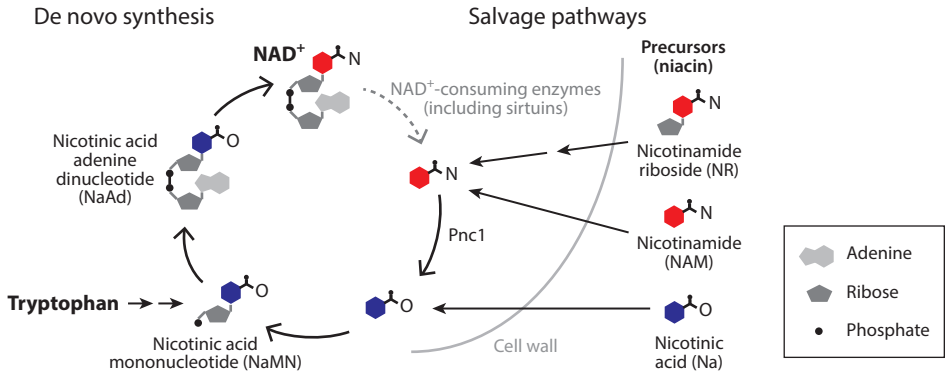

Figure 3

NAD^+ is produced in two ways. The de novo (kynurenine) pathway generates nicotinic acid (Na) from tryptophan. Salvage pathways incorporate precursors [nicotinamide riboside (NR), nicotinamide (NAM), and nicotinic acid (Na)] into nucleotides. The dashed arrow indicates breakdown of NAD^+.

expression of biosynthesis genes when NAD^+ levels fall (11). Other sirtuins modulate metabolic pathways that utilize NAD^+ (57).

REGULATION OF SIRTUIN ACTIVITY IN VIVO

The activity of sirtuins can be regulated by the metabolic state of a cell through changes in NAD^+ availability and by signaling pathways that sense nutrient availability. Moreover, nutrient regimens such as calorie restriction have been proposed to affect sirtuin activity. Such regulation enables sirtuins to transmit information about a cell's metabolic state to the biological processes they influence.

Regulation of Sirtuins by NAD^+ and Related Compounds

Because sirtuins require NAD^+ for deacetylation, intracellular NAD^+ levels influence their activity. For example, in *S. cerevisiae* the activity of the sirtuin Sir2 (silent information regulator 2) is affected by supplemental nicotinic acid or nicotinamide riboside (12) or by genetic perturbations of the enzymes and transporters that maintain intracellular NAD^+ (5, 12, 122). However, sirtuins are not thought to sense the NAD^+:NADH ratio, because the nuclear-cytoplasmic concentration of free NAD^+ is tens to hundreds of times greater than that of free NADH (3, 19).

Although sirtuin activity is enhanced by supplementing cultures with the NAD^+ precursors nicotinic acid or nicotinamide riboside (12), nicotinamide has the opposite effect (13, 79). As a product of the deacetylation reaction (**Figure 2**), nicotinamide inhibits sirtuins by reversing the formation of a covalent intermediate between acetyl-ADP-ribose and acetyllysine (62, 112). Consequently, sirtuin activity can be increased by boosting expression of Pnc1 (7), the enzyme that converts nicotinamide to nicotinic acid (**Figure 3**).

Regulation of Sirtuins by Phosphorylation

In keeping with the expectation that sirtuins are sensitive to nutrient availability, sirtuin activity can be modulated by two pathways that signal nutrient status—the cAMP/protein kinase A (PKA) (47, 67) and target of rapamycin complex 1 (TORC1) (28) pathways. The cAMP/PKA pathway is activated by glucose and the TORC1 pathway is primarily activated by nitrogen-containing

Calorie restriction: restriction of calorie intake without malnutrition; a treatment that can increase life span in a variety of organisms

Sir2 (silent information regulator 2): *S. cerevisiae* Sir2 is the founding sirtuin; its deacetylase activity was discovered 20 years after it was described

molecules such as amino acids. In *S. cerevisiae*, both pathways activate casein kinase 2 (CK2), which phosphorylates a conserved serine in the NAD^+-binding pocket of the Sir2 catalytic domain (28, 67). A phosphomimetic mutation of ScSir2 (S473E) had reduced activity to repress genes, including ribosomal protein genes and *PMA1* (encoding a plasma membrane proton pump). Thus, sirtuin activity can be modulated by altering the phosphorylation state of this serine. Although this serine is highly conserved, its phosphorylation has not been studied in most sirtuins.

Paralogs: related genes in the same organism that arose through duplication of an ancestral gene

Orthologs: related genes in different species that are derived from a common ancestral gene

Sir (silent information regulator) proteins: a group of unrelated proteins in budding yeast that act together to form heterochromatin at telomeres and mating-type loci

Supplemental Material >

Regulation of Sirtuins by Calorie Restriction

Because both sirtuins and calorie restriction influence life span, the effect of calorie restriction on sirtuin activity has been extensively examined. However, there are conflicting reports on this matter. For yeasts, calorie restriction is achieved by lowering the glucose concentration in the growth medium. It was originally proposed that for *S. cerevisiae* under calorie restriction, metabolism shifts from fermentation to respiration, which increases the reoxidation of NADH to NAD^+ and hence elevates NAD^+ levels (86). However, contrary to this model, NAD^+ levels do not change under calorie restriction (7). Instead, calorie restriction increases expression of *PNC1*, which in turn reduces nicotinamide (6), a sirtuin inhibitor. However, another study found that, regardless of how calorie restriction affects Sir2 activity, its effect on life span is independent of Sir2 (64). Thus, studies of how calorie restriction affects sirtuins and how it affects life span have diverged.

SIRTUIN SUBFAMILIES

Most fungal species encode multiple sirtuins, which fall into five subfamilies (**Supplemental Figure 1**). The Sir2/Hst1, Hst2, and Hst3/Hst4 subfamilies have been studied primarily in the model yeast *S. cerevisiae* (the designation Hst derives from "homolog of Sir two"). In contrast, the SirT4 and SirT5 subfamilies, which are not represented in *S. cerevisiae*, have not been examined in fungi. All *S. cerevisiae* sirtuins deacetylate histones, with Sir2 and Hst1 acting at specific genomic loci and the other sirtuins deacetylating histones genome-wide. It is expected that these sirtuins also deacetylate nonhistone proteins, and a few such proteins have been identified. However, additional work is needed to uncover the full set of sirtuin substrates. Here, we describe how representatives of these sirtuin subfamilies, primarily from *S. cerevisiae*, influence gene expression and genome stability through histone deacetylation.

Sir2/Hst1 Subfamily

The Sir2/Hst1 subfamily is orthologous to mammalian SirT1 and is represented by the paralogs Sir2 and Hst1 in *S. cerevisiae*. These two sirtuins arose in a whole-genome duplication that occurred in the *Saccharomyces* lineage. Therefore, both are equally related to Sir2 orthologs in fungi that did not undergo this duplication. Members of this subfamily deacetylate histones, particularly histone H4 Lys-16 (H4K16) and histone H3 Lys-9 (H3K9). They are recruited by DNA-binding proteins to specific genomic locations, where they generate condensed, repressive chromatin.

Heterochromatin formation. In both *S. cerevisiae* and *Schizosaccharomyces pombe*, Sir2 contributes to the formation of heterochromatin that extends over thousands of base pairs and represses transcription. However, distinct mechanisms are used in the two species.

In *S. cerevisiae*, Sir2 and other Sir proteins form heterochromatin at telomeres and silent mating-type loci. At telomeres, this SIR chromatin is thought to stabilize the ends of chromosomes. At the silent mating-type loci, it confers cell-type identity by repressing extra copies of cell-type-specific transcription factors. The assembly of SIR chromatin occurs through the

cooperation of Sir2 and a nucleosome-binding protein, Sir3 (55, 91, 110). First, the Sir proteins are recruited to silenced loci through DNA-binding proteins. Next, Sir2 deacetylates adjacent nucleosomes. Multiple histone lysines are affected, but H4K16 is a preferred and important target (59). These deacetylated nucleosomes are then bound by Sir3, which has higher affinity when H4K16 is unacetylated (8, 102). Finally, the nucleosome-associated Sir3 attracts additional Sir2 molecules, which then deacetylate nucleosomes farther from the recruitment site. Multiple cycles of deacetylation and nucleosome binding enable the formation of an extended chromatin structure. A similar mechanism generates silenced chromatin in related budding yeast, including *Candida glabrata* (25), *Kluyveromyces lactis* (52), and *Torulaspora delbrueckii* (33).

In *S. pombe*, Sir2 generates heterochromatin through a distinct mechanism that resembles mammalian heterochromatin formation. This heterochromatin occurs at centromeres, silent mating-type loci, and telomeres (39, 114). The contribution of Sir2 is best understood at centromeres (2, 18) and includes two assembly steps: nucleation and spreading. In the nucleation step, deacetylation of H3K14 and H3K9 by Sir2 creates a permissive environment for recruitment and methylation by Clr4. Clr4 methylates H3K9 and is an ortholog of mammalian SUV39. Methylated H3K9 is then bound by Swi6, a structural component of heterochromatin that is an ortholog of HP1. In the spreading step, Sir2 acts cooperatively with another deacetylase, Clr3, to facilitate H3K9 methylation by Clr4 and subsequent binding of Swi6.

In *Candida albicans*, Sir2 also contributes to silencing at telomeres (4) but not at centromeres or silent mating-type loci, which are absent. Although Sir2 deacetylates histones (42, 107, 109), other mechanistic details of silencing at telomeres are unknown. *C. albicans* lacks orthologs of the *S. cerevisiae* Sir proteins and *S. pombe* Clr4 and Swi6 heterochromatin proteins.

Suppression of rDNA recombination. Discussions of Sir2-mediated silencing in *S. cerevisiae* typically mention three genomic loci: telomeres, mating-type loci, and ribosomal DNA (rDNA). However, rDNA silencing is mechanistically distinct, as it does not involve Sir3 or Sir4. Moreover, although the action of Sir2 at the rDNA locus can be evaluated by measuring transcriptional silencing of reporter genes (121), the main biological role of Sir2 is to suppress recombination (48). This is critical because the repetitive rDNA array is a major site of genomic instability.

In *S. cerevisiae*, the rDNA locus consists of ~150 tandem repeats that encode ribosomal RNA (rRNA). Maintaining a consistent number of these nearly identical repeats is critical for producing sufficient ribosomes, and therefore multiple mechanisms exist to suppress recombination and to amplify rDNA arrays that become too short. A key feature in these mechanisms is a recombination hot spot that coincides with a unidirectional replication fork barrier at the end of the rRNA gene (72). This barrier prevents DNA replication from occurring in the opposite direction to transcription. However, the barrier also causes stalled replication forks, which can lead to double-strand breaks that in turn trigger recombination. Typically, recombination does not alter the number of repeats because sister chromatids are kept in register by cohesin (73). However, if cohesin is displaced, unequal sister chromatid recombination can lead to amplification of the array (71). Such displacement occurs when a nearby polymerase II promoter is transcribed. Ordinarily, Sir2 represses this promoter (81). However, when the rDNA array becomes critically short, a feedback mechanism represses transcription of the *SIR2* gene and hence availability of Sir2 to suppress recombination (58, 101). Specifically, the UAF transcription factor, which normally binds to the rRNA promoter, is in excess due to the low number of rDNA repeats. It is therefore available to bind to and repress the *SIR2* promoter. Sir2 recruitment is also regulated by nutrient availability. When nutrient levels are low, the target of rapamycin (TOR) pathway is inhibited, which increases Sir2 association with the rDNA (49). In addition to influencing the distribution of cohesin, a second way in which Sir2 influences recombination is by influencing initiation of DNA replication

rDNA (ribosomal DNA): DNA sequences that code for ribosomal RNA; multiple rDNA genes are typically arranged in tandem head-to-tail arrays

Recombination: rearrangement and exchange of DNA strands, often at homologous sequences

and hence the number of stalled forks. This is achieved through Sir2-mediated repression of a second polymerase II promoter adjacent to the origin of replication within each repeat (38).

The role of Sir2 in regulating rDNA recombination has received little attention in other species. Sir2 orthologs are associated with the rDNA in *S. pombe* (135), *C. albicans* (42, 109), and *Clavispora lusitaniae* (43). Curiously, in *C. albicans*, although CaSir2 can silence transcription within the rDNA repeats, it may not repress recombination (41).

Secondary metabolite: small molecule that is not a core metabolite but helps an organism kill or communicate with other species or scavenge nutrients

Promoter-targeted repression. In *S. cerevisiae*, Hst1 is a paralog of Sir2. However, rather than generating extended chromatin structures, Hst1 acts locally at target promoters to repress transcription. Target genes include midsporulation genes and NAD^+ biosynthesis genes (11, 138). Hst1 is recruited to these promoters as part of the SUM1C repressor complex, which contains the DNA-binding protein Sum1 (138). A similar complex containing Sum1 and Hst1 has also been described in related species, including *C. glabrata* (93, 103) and *K. lactis* (53, 57).

In other fungal species, sirtuins of the Sir2/Hst1 subfamily can also carry out promoter-specific repression. For example in *Aspergillus nidulans*, SirA represses genes required to produce secondary metabolites (61, 117). However, it is not known how SirA is recruited to its target loci, as the DNA-binding protein Sum1 is only found in the Saccharomycetaceae family of budding yeasts.

Hst2 Subfamily

The Hst2 subfamily is orthologous to mammalian SirT2 and is represented by Hst2 in *S. cerevisiae*. The best-characterized role of *S. cerevisiae* Hst2 is to promote chromosome condensation in preparation for mitosis. This process begins with activation of the kinase Aurora B (Ipl1 in *S. cerevisiae*), which regulates many aspects of mitosis. For chromosome condensation, a key target of the Aurora B kinase is H3S10 (56). Phosphorylated H3S10, in turn, recruits ScHst2 to nucleosomes, where it then deacetylates H4K16 (130, 131). The deacetylated tail of histone H4 is thought to interact with the acidic patch of a neighboring nucleosome, thereby promoting fiber condensation. This short-range mode of chromosome condensation is independent of axial compaction mediated by condensin, which occurs later in anaphase (76). The interaction of ScHst2 with nucleosomes occurs predominantly at G2/M phase, in part because this is when H3S10 is phosphorylated. In addition, the interaction requires phosphorylation of ScHst2 (S320 or S324), which is enhanced at G2/M phase (63). This interaction between phosphorylated H3S10 and ScHst2 is mediated by the 14–3–3 protein Bmh1 (63). It is not known whether Hst2 also condenses chromosomes in other fungal species.

One puzzling aspect of Hst2 is that it is localized to the cytoplasm in *S. cerevisiae* (105) as well as *S. pombe* and *C. albicans* (31, 140), but its function in chromosome compaction occurs in the nucleus. Moreover, the *S. cerevisiae* nucleus does not break down during mitosis. A potential explanation is that Hst2 shuttles in and out of the nucleus (31, 132) and thus could be recruited to chromosomes during its time in the nucleus.

Another unresolved issue is the extent to which Hst2 orthologs are also targeted to specific genomic locations. For example, ScHst2 affects transcription of the subtelomeric *FLO10* gene and is proposed to be recruited through the transcription factor Sfl1 (51). Similarly, SpHst2 represses genes involved in membrane function and transport (31). It is also expected that fungal Hst2 orthologs deacetylate nonhistone proteins, particularly in the cytoplasm.

Hst3/Hst4 Subfamily

The Hst3/Hst4 subfamily does not have a mammalian ortholog. In *S. cerevisiae*, it is represented by Hst3 and Hst4, which have overlapping roles. Other species, including *C. albicans* and *S. pombe*,

encode a single member of this sirtuin subfamily. The best-studied function of Hst3/Hst4 sirtuins is the genome-wide deacetylation of histone H3K56, which is necessary to maintain genome stability. H3K56 is acetylated in newly synthesized histones before they associate with DNA, and thus H3K56Ac is high during S phase (97). This modification is removed during G2/M by Hst3 and Hst4 (21, 94). Within the nucleosome structure, H3K56 falls at the points where DNA enters and exits. Unacetylated Lys-56 interacts electrostatically with the DNA backbone, whereas acetylation eliminates this interaction and allows a segment of DNA to peel off the nucleosome. This loosening of nucleosomes by H3K56Ac is thought to facilitate chromatin disassembly and reassembly during DNA replication and repair reactions and to make chromatin more permissive to transcription initiation. Consequently, too much or too little acetylation at H3K56 leads to genome instability. Accordingly, *S. cerevisiae hst3*Δ *hst4*Δ cells display hallmarks of DNA damage. Similarly, in *S. pombe*, deletion of *SpHST4* or mutation of H3K56 resulted in fragmented DNA and sensitivity to DNA-damaging agents (31, 40, 50, 74). And in *C. albicans*, deletion of *CaHST3* resulted in H3K56 hyperacetylation, increased sensitivity to genotoxic agents, and loss of cell viability (137).

Gene duplication: production of extra copies of a gene; may be associated with segmental or whole-genome duplication

To produce an oscillation of H3K56Ac with the cell cycle, Hst3 and Hst4 protein levels are regulated. In *S. cerevisiae*, Hst3 peaks before Hst4. Its short half-life is due to two degrons in its C terminus. Once these elements are phosphorylated, they are recognized by the ubiquitin ligase Cdc4, leading to degradation by the proteasome (27, 32, 126). Several kinases have been implicated in phosphorylating the Hst3 degrons, including the cyclin-dependent kinase Cdk1 (27) and DNA-damage-activated kinases Mec1 and Mck1 (32, 126). The latter allow H3K56Ac to accumulate at genomic loci undergoing repair. Hst4 stability may be similarly regulated, but it has not been studied extensively. In addition, Hst4 is regulated by the TOR pathway (136). TORC1 signaling ceases upon nitrogen starvation, alleviating its inhibition of the phosphatase Sit4. The activation of Sit4 results in the redistribution of Hst4 from the cytoplasm to the nucleus (136), although it is unclear whether Sit4 directly dephosphorylates Hst4.

An important unresolved issue is how the expression patterns and functions of *S. cerevisiae* Hst3 and Hst4 differ and what advantage is conferred by having two related paralogs. As *S. pombe* and *C. albicans* each encode a single sirtuin from the Hst3/Hst4 subfamily, it is not essential to have two forms of the protein. Another issue is whether Hst3/Hst4 proteins also target specific genomic loci. One example of such behavior is SirE from *A. nidulans*, which represses metabolic genes as the fungus transitions to stationary phase (60).

SirT4 and SirT5 Subfamilies

Some fungi encode homologs of mammalian SirT4 and SirT5 (**Supplemental Figure 1**), but their functions are unknown. In mammals, both SirT4 and SirT5 are mitochondrial proteins. SirT5 desuccinylate and demalonylate proteins involved in metabolism and reactive oxygen species detoxification (77). The enzymology of SirT4 is less understood, but it regulates adipocyte differentiation and insulin production (84, 139).

Supplemental Material >

EVOLUTION OF FUNGAL SIRTUINS

Like other enzyme families, the sirtuin family has expanded over evolutionary time through gene duplications followed by diversification of substrate specificity. Ancient duplications in the eukaryotic lineage produced the five subfamilies discussed above. More recent duplications gave rise to paralogs, such as Sir2 and Hst1 in *S. cerevisiae*. On a shorter timescale, a specific sirtuin may gain or lose deacetylation targets, including specific genomic targets or protein substrates.

Together, these processes enable sirtuins to serve as rewiring points that allow organisms to evolve novel responses to low NAD^+ stress by bringing relevant biological processes under the control of sirtuins.

Diversification of Sirtuins Through Gene Duplication and Loss

How sirtuins diverge after gene duplication has been examined in the case of Sir2 and Hst1 (44, 53, 99), which arose through a whole-genome duplication in the Saccharomycetaceae family of budding yeasts. Two paths by which duplicated genes can diverge are neofunctionalization, in which one duplicate gains a new function, and subfunctionalization, in which functions of the ancestral protein are partitioned between the duplicates. Sir2 and Hst1 likely subfunctionalized because the nonduplicated Sir2/Hst1 protein from *K. lactis* behaves like both ScSir2 (generates heterochromatin at mating-type loci and telomeres) and ScHst1 (represses specific promoters) (10, 53). Moreover, this subfunctionalization occurred through a duplication, degeneration, and complementation route, as Sir2 and Hst1 acquired complementary mutations that reduced their association with either the SIR or SUM1C repressive complexes (44). This type of process may also have occurred after other sirtuin duplications.

A separate duplication of Sir2 occurred in the CUG-Ser1 clade of budding yeasts (109), which includes *C. albicans*. The resulting paralogs have also been called Sir2 and Hst1, which is unfortunate because there is not a biologically meaningful correspondence between the Sir2 proteins or the Hst1 proteins in *C. albicans* and *S. cerevisiae*. Interestingly, in the CUG-Ser1 clade, the Sir2 paralog has been lost multiple times, whereas Hst1 has been uniformly retained (109). This pattern of loss implies that the function of Hst1 is advantageous, but it is unclear what this advantageous function is. The only known function for Hst1 in *C. lusitaniae*, which lacks Sir2, is at the rDNA array (43, 68). However, in *C. albicans*, it is Sir2 and not Hst1 that acts at the rDNA (41, 42), raising the possibility that sirtuins can regain ancestral functions to compensate for gene loss (109).

Shifts in Sirtuin Targets

After sirtuins arise through duplication, they evolve with their host species and can acquire or lose substrates. This may occur when their interactions with adaptor proteins shift, as in the case described above for Sir2 and Hst1 from the Saccharomycetaceae family (44). Another mechanism contributing to substrate shifts is a change in the target. For example, in the case of genes repressed by sirtuins, a promoter may gain or lose a recognition site for a DNA-binding protein that recruits a sirtuin. This process can result in orthologous sirtuins repressing different gene sets in different species, as is seen for the genes repressed by SUM1C in *S. cerevisiae* compared to *K. lactis* (57) and *C. glabrata* (103).

Sirtuins as Rewiring Points

Because sirtuins have a fixed requirement for NAD^+ but shifting deacetylation targets, we have proposed that sirtuins can serve as rewiring points (57). As sirtuins acquire new targets, the biological processes associated with these targets will become responsive to intracellular NAD^+ levels. Thus, organisms can evolve potentially adaptive responses to low NAD^+ stress by bringing new processes under the control of sirtuins. In support of this rewiring hypothesis, we found that distinct sets of genes are regulated by the SUM1C repressor in *S. cerevisiae* and *K. lactis* (57). For example, only *K. lactis* SUM1C regulates genes that detoxify arsenic. Thus, increased resistance to arsenic is predicted to be a distinct response of *K. lactis* to low NAD^+. Also consistent with this hypothesis is the observation that the sets of SUM1C-regulated genes in *S. cerevisiae* and *K. lactis* contain fewer broadly conserved genes than expected (57).

ROLE OF SIRTUINS IN FUNGAL RESPONSE TO LOW NUTRIENTS

Fungi occupy diverse ecological niches and utilize a variety of nutrients. Once these nutrients are depleted, fungi must deploy survival strategies to persist until more nutrients are obtained. These strategies include metabolic shifts to consume alternate nutrients, filamentous growth to access distant nutrients, and sporulation for dispersal and long-term survival (reviewed in 54, 134). Environmental stress also induces fungi to secrete secondary metabolites that modify the environment (69, 128). Moreover, in the case of pathogens, these strategies are adapted to promote invasion and survival within host tissue. Often, these survival strategies are only activated upon nutrient depletion or other stresses, and cells integrate information about environmental conditions before committing to these developmental transitions. As NAD^+ sensors, sirtuins contribute to these commitment decisions.

Filamentous growth: growth pattern of fungi in which cells are elongated and may be connected in chains

Sporulation: production of haploid spores through meiosis, often under unfavorable environmental conditions

Maintaining NAD^+ Pools

Cells respond to low NAD^+ levels by upregulating genes involved in NAD^+ synthesis and salvage pathways. In *S. cerevisiae*, Hst1 participates in this induction (11). When intracellular NAD^+ levels fall, Hst1 is thought to be the first sirtuin affected due to its higher K_m for NAD^+ (11). Reduced deacetylation by Hst1 leads to derepression of SUM1C target genes, including genes encoding NAD^+ biosynthesis enzymes and niacin transporters. Higher expression of these genes increases NAD^+ synthesis and niacin uptake. Once NAD^+ levels are restored, Hst1 regains its deacetylase activity and again represses its target genes. This type of feedback mechanism is also seen in *C. glabrata* and *K. lactis* (57, 92). However, both these species lack the *BNA* genes required for de novo NAD^+ synthesis, and therefore reduced sirtuin activity leads to induction of genes encoding niacin transporters and salvage pathway enzymes. It is not known whether sirtuins contribute to NAD^+ homeostasis in fungal species that lack the SUM1C repressor complex.

Metabolic Shifts

When fungi deplete favored nutrients, such as glucose, they must adjust their metabolic flux to utilize other nutrients. For example, when *S. cerevisiae* runs out of glucose and other sugars, it undergoes a diauxic shift, switching from fermentation of glucose to respiration of the ethanol and acetate produced during fermentation. Some genes that are upregulated during the diauxic shift are repressed by Sir2 and Hst1 (57, 83), suggesting that lower NAD^+ may be one signal that promotes the shift.

A different role for a sirtuin in a metabolic shift is seen for the mold *A. nidulans*. The sirtuin SirE, which is homologous to ScHst3 and ScHst4, binds to the promoters of primary metabolic genes, including those involved in the glycolytic pathway, tricarboxylic acid cycle, and cell wall synthesis (60). In the exponential growth phase, the promoters of these genes are acetylated and transcription is high. However, during stationary phase when nutrients are limited, these promoters are deacetylated by SirE and transcription is repressed. It remains unclear how the deacetylase activity of SirE is regulated according to the growth phase.

Sporulation

For yeasts, sporulation is a response of diploid cells to starvation in which the cell undergoes meiosis and then encases each of the four daughter nuclei in a stress-resistant spore wall. Sporulation is induced by a poor carbon source. However, during the early phases of sporulation, cells can return to mitotic growth if they are refed. In contrast, once cells begin the first meiotic division, they are committed to sporulation, even if nutrients are restored (134). Thus, commitment to sporulation

requires a sustained nutrient starvation signal, and in *S. cerevisiae* low NAD^+ may enhance such a signal. This is because the commitment point corresponds with the expression of the transcription factor Ndt80, which is regulated by the SUM1C repressor complex (106). The *NDT80* promoter serves as a genetic switch in which a single sequence, called the midsporulation element, can associate with either a repressor or an activator, but not both. The repressor is SUM1C, and the activator is Ndt80 itself. When SUM1C dominates, *NDT80* is repressed, and the switch is turned off. However, as sporulation proceeds, Sum1p is phosphorylated, resulting in the release of Hst1p and the weakening of repression (22). Once a little Ndt80 protein is produced, it displaces Sum1 from the promoter, increasing transcription and turning the genetic switch on. The cell then commits to sporulation. Thus, regulatory mechanisms, such as low intracellular NAD^+, that reduce repression by SUM1C increase the probability of commitment to sporulation.

Secondary Metabolite Production

Fungi, particularly molds, are well known for producing secondary metabolites in response to adverse environmental conditions. Secondary metabolites are compounds that are not part of central metabolism but instead promote the organism's survival by protecting it from environmental stresses or diminishing competitor species. For example, melanin protects *Cryptococcus neoformans* against UV radiation and oxidative stress, and penicillin kills bacteria that may compete with the mold *Penicillium rubens*. In some cases, secondary metabolite production is regulated by sirtuins, suggesting that it could be modulated by NAD^+ availability or other factors that influence sirtuin activity.

A. nidulans is an important model filamentous fungus that is renowned for its secondary metabolite production, including the antibiotic penicillin G and the toxin sterigmatocystin (17, 95). Interestingly, the genes involved in penicillin G and sterigmatocystin biosynthesis are regulated by the sirtuin SirA, which associates with their promoters and represses them via H4K16Ac deacetylation (60, 117). Therefore, *A. nidulans* may factor NAD^+ availability into the decision to produce these metabolites, and artificially inhibiting SirA could be a strategy to induce secondary metabolites (116, 117).

The budding yeast *K. lactis* produces the secondary metabolite pulcherrimin (75), which binds iron. As iron is essential for the growth of microorganisms, iron sequestration by pulcherrimin antagonizes microbes that lack a pulcherrimin transporter (119, 129). The two biosynthesis genes, *PUL1* and *PUL2*, are repressed by SUM1C and induced in its absence. Moreover, pulcherrimin production is increased when NAD^+ is low (57).

SIRTUINS AND LIFE SPAN

Arguably the most famous biological role of sirtuins is to slow aging, and this role was first identified in *S. cerevisiae* (66). Yeasts have served as model organisms for aging studies with the use of two assays. Replicative life span is measured as the number of times a mother cell buds before senescing, and chronological life span is determined as the length of time a nondividing cell remains viable after nutrients are depleted. During replicative aging, damaged cellular components are preferentially retained in the mother cell, leading to its ultimate senescence but resetting the age of the daughter bud. These damaged components include extrachromosomal DNA (118), oxidized and misfolded proteins (1), and dysfunctional mitochondria (98), which cumulatively degrade cellular health. Old mother cells also have reduced levels of Sir2 and Hst1 (24, 37) and unstable rDNA (88). During chronological aging, metabolism is reconfigured and slowed (35). *S. cerevisiae* cells first shift from fermentation of glucose to respiration of ethanol and acetate (diauxic shift) and eventually utilize storage molecules such as trehalose and glycogen. A successful

transition requires reversal of the glycolytic pathway, which consumes sugar, to gluconeogenesis, which produces sugar for storage.

Replicative life span is decreased in the absence of Sir2 and increased by extra copies of *SIR2* in *S. cerevisiae* (66), as well as *C. albicans* (45) and *C. neoformans* (14). In *S. cerevisiae*, Sir2 primarily influences replicative aging through its suppression of unequal sister chromatid recombination within the rDNA, based on observations that loss of Sir2 has only a modest effect on life span when the Sir2-regulated promoter within the rDNA is removed (111). Moreover, the rDNA is a major QTL (quantitative trait locus) that influences aging (78, 124). Originally, the aging effect of rDNA recombination was thought to be the production of extrachromosomal rDNA circles, which accumulate in the mother cell and may sequester replication and repair proteins away from genomic DNA (118). However, aging can occur without accumulation of extrachromosomal rDNA circles, leading to the hypothesis that it is the instability of rDNA that leads to aging by triggering checkpoints and DNA repair (46). Similarly, in *C. albicans* deletion of *CaSIR2* did not result in the accumulation of extrachromosomal rDNA molecules (41). A second way Sir2 impacts replicative life span is by promoting asymmetric segregation of toxic components through its effect on retrograde actin cable flow (1). In particular, a chaperone required for actin folding, CCT, is acetylated and less active in the absence of Sir2 (89). However, it was not determined whether CCT is a direct substrate of Sir2. In *C. albicans*, CaSir2 also promotes asymmetric distribution of oxidatively damaged proteins during cell division (45). A third way Sir2 impacts replicative life span is by maintaining appropriate H4K16 acetylation levels and subtelomeric silencing (24). However, it remains unknown why elevated H4K16 acetylation shortens life span.

Chronological life span is influenced by Sir2 in the opposite direction of replicative life span. Loss of Sir2 increases chronological life span (34). One way that Sir2 influences chronological aging is by deacetylating Pck1 (87), a key enzyme required for gluconeogenesis but not glycolysis. Deacetylation reduces the activity of Pck1, and hence Sir2 inhibits gluconeogenesis. Consequently, in the absence of Sir2, stationary phase cells consume ethanol and acetate more quickly (20, 34), which is beneficial because acetate in the spent medium contributes to cell death. In addition, increased gluconeogenesis produces more of the storage molecule trehalose.

With an eye to extending life span pharmacologically, considerable effort has focused on interventions that boost Sir2 activity and hence replicative life span. These interventions include compounds that act directly on sirtuins, manipulation of intracellular NAD^+ levels, inhibition of signaling pathways that sense nutrients, and calorie restriction. A naturally occurring direct regulator of sirtuin activity is the inhibitor nicotinamide, and indeed supplemental nicotinamide reduces yeast replicative life span (13). Nicotinamide levels can be reduced by increasing Pnc1, the enzyme that converts nicotinamide to nicotinic acid (**Figure 3**). Indeed, upregulating Pnc1 increases replicative life span (6). The second type of intervention, increasing intracellular NAD^+ levels, can be achieved by supplementing the growth medium with niacin. Such treatment increases replicative life span (12). The third intervention is to inhibit the TORC1 and cAMP/PKA pathways that signal the presence of nutrients. Indeed, inhibition of either pathway increases life span (65). However, it is not clear whether these pathways act through sirtuins. One potential link is that the casein kinase CK2, which is activated by both pathways, phosphorylates Sir2 and reduces its activity (28, 67). Another link is that inhibition of these pathways leads to activation of transcription factors Msn2 and Msn4, which in turn upregulate *PNC1*, thereby decreasing nicotinamide (100). The final intervention to increase life span is calorie restriction. For yeasts, this is achieved by lowering glucose in the growth medium, which does increase replicative life span. However, it is controversial whether calorie restriction activates sirtuins, as Sir2 is not required for the effects of calorie restriction in replicative aging (127) or chronological aging

(120). Nevertheless, calorie restriction does reduce signaling through TORC1, and hence leads to upregulation of Pnc1 and phosphorylation of Sir2.

Virulence traits: factors, including molecules and cellular processes, that enable microbial pathogens to achieve robust infection

SIRTUINS AND VIRULENCE TRAITS IN PATHOGENIC FUNGI

In pathogenic fungi, sirtuins have evolved to influence virulence traits that help the organism respond appropriately to changes in the host environment.

Candida glabrata

C. glabrata is the second-most common cause of human yeast infections, after *C. albicans*. Although it is more closely related to *S. cerevisiae* than other pathogenic yeasts are (96) (**Figure 1**), it has gained traits necessary for a commensal lifestyle (108). Compared to *S. cerevisiae*, *C. glabrata* is more adhesive to surfaces and has higher resistance to drugs and stresses such as reactive oxygen species and nutrient starvation. *C. glabrata* is unable to synthesize NAD^+ via the de novo pathway, so environmental niacin availability dramatically affects intracellular NAD^+ levels and thus sirtuin activity (93). In low niacin, inhibition of both CgSir2 and CgHst1 induces expression of genes that promote virulence.

CgSir2 regulates the expression of subtelomeric genes in response to changes in NAD^+ levels (29). Some of these genes (*EPA*) encode adhesins that mediate adherence to host epithelial cells, an important virulence trait (23, 26). When *C. glabrata* enters the urinary tract, which is a niacin-poor environment, intracellular NAD^+ level drops, compromising the activity of CgSir2. The resulting loss of subtelomeric silencing permits expression of *EPA* genes, leading to increased adherence to epithelial cells and promoting urinary tract infection (29).

The CgSUM1C repressor, containing Hst1, is also important for regulating virulence traits in response to NAD^+ levels (103). In the absence of CgHst1, *C. glabrata* is more resistant to fluconazole, due to increased expression of the multidrug transporter *CDR1*, and more resistant to hydrogen peroxide, due to increased expression of catalase *CTA1*. In contrast, in *S. cerevisiae* the deletion of *ScHST1* did not affect susceptibility to fluconazole or hydrogen peroxide. This finding suggests that CgSUM1C gained the ability to regulate *CDR1* and *CTA1* in the *C. glabrata* lineage, which may have helped it adapt as a human commensal.

Candida albicans

C. albicans is a major opportunistic human fungal pathogen that diverged from *S. cerevisiae* around 240 million years ago (115). In healthy individuals, it is often a benign member of the skin and mucosal microbiome. However, in people with compromised immune systems, *C. albicans* can switch to a pathogenic state and causes severe superficial and life-threatening systemic infections. One important virulence trait is the ability to switch between yeast and filamentous forms. Yeast cells can disseminate throughout the body, whereas elongated hyphal cells can penetrate host tissue.

CaSir2 influences filamentous growth. An early study suggested that inactivation of CaSir2 increased filamentous growth in both solid and liquid media (104). However, these findings have not been reproduced, and a recent study reached the opposite conclusion, demonstrating that formation of hyphae was reduced in the absence of CaSir2 (140). Consistent with this finding, other studies showed that *CaSIR2* is upregulated as hyphae develop and that garlic compounds inhibit yeast-to-hypha transition by downregulating *SIR2* (70, 85, 90). Importantly, the regulation of hypha formation by Sir2 is dependent on its deacetylase activity (140), suggesting that if CaSir2 is compromised by low intracellular NAD^+, cells will be less likely to filament. Thus, information

about the cell's metabolic state may influence the decision to filament. In the future, it will be important to identify the proteins or genomic loci whose deacetylation by CaSir2 influences filamentous growth.

Another potential contribution of CaSir2 to virulence is its regulation of the subtelomeric *TLO* genes (4), which encode variants of a mediator subunit involved in transcriptional regulation. The action of CaSir2 in subtelomeric regions leads to cell-to-cell variation in the expression of *TLO* genes, which may generate phenotypic diversity among cells and hence promote adaptation to environmental changes.

CaHst3 has been considered as an antifungal drug target because it is critical for viability and differs from human sirtuins (137). CaHst3 retains the well-conserved function of Hst3/Hst4 subfamily members in maintaining genome stability by deacetylating H3K56. In addition, loss of CaHst3 induces hyperfilamentation, but with abnormally shaped filaments; sensitizes cells to genotoxic and antifungal agents; and impairs *C. albicans* infection in a mouse model (123, 137).

Cryptococcus neoformans

C. neoformans is a pathogenic yeast that causes severe infections in immunocompromised people, especially those suffering from AIDS. Unlike the other species discussed here, it belongs to the phylum Basidiomycota. CnSir2 promotes virulence in two ways. First, lack of Sir2 causes a growth defect that results in reduced virulence in both murine and wax moth (*Galleria mellonella*) infection models without modulating virulence traits (9, 14). Second, because CnSir2 modulates replicative life span and old cells are more virulent (16), studies have examined how drugs that alter Sir2 activity affect virulence (14). Treatment with Sir2 agonists, which prolonged replicative life span, decreased virulence of *C. neoformans* in *G. mellonella*, whereas treatment with Sir2 antagonists had the opposite effect. However, the outcome of these treatments varied with the strain (15), and further investigation is needed.

Magnaporthe oryzae

M. oryzae infects cultivated rice and other grains and is one of the most significant agricultural pathogens (133). To establish an infection, *M. oryzae* must withstand reactive oxygen species produced by the plant (36). The sirtuin MoSir2 (MGG_10267), which belongs to the Hst2 subfamily, assists in this defense by indirectly promoting the expression of the superoxide dismutase Sod1. MoSir2 deacetylates the transcriptional repressor Jmjc, thereby blocking its association with the promoter of *MoSOD1* and enabling transcription. MoSir2 levels are regulated via ubiquitination (82). The E3 ubiquitin ligases Grr1 and Upl3 directly interact with MoSir2 and have opposite roles in regulating MoSir2 levels. In the absence of oxidative stress, Grr1 contributes to MoSir2 turnover. In contrast, in response to oxidative stress, Upl3 promotes MoSir2 accumulation.

SUMMARY POINTS

1. Sirtuins deacetylate histones, which represses transcription and impacts genome stability.
2. Because sirtuins are NAD^+-dependent deacetylases, their activity is sensitive to intracellular NAD^+ availability.
3. NAD^+ is a critical metabolite that must be maintained in sufficient quantities and whose levels could be affected by abrupt shifts in metabolic flux.

4. The regulation of sirtuins by NAD^+ levels and nutrient-sensing signaling pathways allows them to transmit information about a cell's metabolic state on to the biological processes they influence.
5. The sirtuin family has expanded over evolutionary time through gene duplications followed by diversification of substrate specificity.
6. Sirtuins serve as rewiring points that allow organisms to evolve novel responses to low NAD^+ stress by bringing relevant biological processes under the control of sirtuins.
7. Fungi integrate information about environmental conditions before committing to metabolic and developmental transitions. Sirtuins contribute to these commitment decisions by sensing NAD^+ levels.
8. In pathogenic fungi, sirtuins influence virulence traits that help the organism respond appropriately to changes in the host environment.

FUTURE ISSUES

1. To understand the specific functions of each sirtuin, its deacetylation targets should be elucidated. These include the genomic loci where it acts and the nonhistone proteins it deacetylates.
2. To understand how sirtuins associate with their genomic and protein targets, their interacting partners should be identified.
3. To understand how sirtuin activity is regulated, information is needed on (*a*) how nutrient fluctuation and other environmental changes affect intracellular NAD^+ levels and (*b*) how sirtuin activity responds to fluctuating NAD^+ levels.
4. Additional regulatory mechanisms should also be investigated, including phosphorylation, subcellular localization, and protein turnover of sirtuins.
5. To understand how sirtuins serve as rewiring points, the targets of orthologous sirtuins should be compared in multiple species.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Aguilaniu H, Gustafsson L, Rigoulet M, Nystrom T. 2003. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* 299:1751–53
2. Alper BJ, Job G, Yadav RK, Shanker S, Lowe BR, Partridge JF. 2013. Sir2 is required for Clr4 to initiate centromeric heterochromatin assembly in fission yeast. *EMBO J.* 32:2321–35

3. Anderson KA, Madsen AS, Olsen CA, Hirschey MD. 2017. Metabolic control by sirtuins and other enzymes that sense NAD^+, NADH, or their ratio. *Biochim. Biophys. Acta Bioenerg.* 1858:991–98
4. Anderson MZ, Gerstein AC, Wigen L, Baller JA, Berman J. 2014. Silencing is noisy: Population and cell level noise in telomere-adjacent genes is dependent on telomere position and Sir2. *PLOS Genet.* 10:e1004436
5. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Cohen H, et al. 2002. Manipulation of a nuclear NAD^+ salvage pathway delays aging without altering steady-state NAD^+ levels. *J. Biol. Chem.* 277:18881–90
6. Anderson RM, Bitterman KJ, Wood JG, Medvedik O, Sinclair DA. 2003. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. *Nature* 423:181–85
7. Anderson RM, Latorre-Esteves M, Neves AR, Lavu S, Medvedik O, et al. 2003. Yeast life-span extension by calorie restriction is independent of NAD fluctuation. *Science* 302:2124–26
8. Armache KJ, Garlick JD, Canzio D, Narlikar GJ, Kingston RE. 2011. Structural basis of silencing: Sir3 BAH domain in complex with a nucleosome at 3.0 Å resolution. *Science* 334:977–82
9. Arras SDM, Chitty JL, Wizrah MSI, Erpf PE, Schulz BL, et al. 2017. Sirtuins in the phylum Basidiomycota: a role in virulence in *Cryptococcus neoformans*. *Sci. Rep.* 7:46567
10. Astrom SU, Kegel A, Sjostrand JO, Rine J. 2000. *Kluyveromyces lactis* Sir2p regulates cation sensitivity and maintains a specialized chromatin structure at the cryptic α-locus. *Genetics* 156:81–91
11. Bedalov A, Hirao M, Posakony J, Nelson M, Simon JA. 2003. NAD^+-dependent deacetylase Hst1p controls biosynthesis and cellular NAD^+ levels in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 23:7044–54
12. Belenky P, Racette FG, Bogan KL, McClure JM, Smith JS, Brenner C. 2007. Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD^+. *Cell* 129:473–84
13. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA. 2002. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast Sir2 and human SIRT1. *J. Biol. Chem.* 277:45099–107
14. Bouklas T, Jain N, Fries BC. 2017. Modulation of replicative lifespan in *Cryptococcus neoformans*: implications for virulence. *Front. Microbiol.* 8:98
15. Bouklas T, Masone L, Fries BC. 2018. Differences in sirtuin regulation in response to calorie restriction in *Cryptococcus neoformans*. *J. Fungi.* 4:26
16. Bouklas T, Pechuan X, Goldman DL, Edelman B, Bergman A, Fries BC. 2013. Old *Cryptococcus neoformans* cells contribute to virulence in chronic cryptococcosis. *mBio* 4:e00455-13
17. Brown DW, Yu JH, Kelkar HS, Fernandes M, Nesbitt TC, et al. 1996. Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*. *PNAS* 93:1418–22
18. Buscaino A, Lejeune E, Audergon P, Hamilton G, Pidoux A, Allshire RC. 2013. Distinct roles for Sir2 and RNAi in centromeric heterochromatin nucleation, spreading and maintenance. *EMBO J.* 32:1250–64
19. Canelas AB, van Gulik WM, Heijnen JJ. 2008. Determination of the cytosolic free NAD/NADH ratio in *Saccharomyces cerevisiae* under steady-state and highly dynamic conditions. *Biotechnol. Bioeng.* 100:734–43
20. Casatta N, Porro A, Orlandi I, Brambilla L, Vai M. 2013. Lack of Sir2 increases acetate consumption and decreases extracellular pro-aging factors. *Biochim. Biophys. Acta Mol. Cell Res.* 1833:593–601
21. Celic I, Masumoto H, Griffith WP, Meluh P, Cotter RJ, et al. 2006. The sirtuins Hst3 and Hst4p preserve genome integrity by controlling histone H3 lysine 56 deacetylation. *Curr. Biol.* 16:1280–89
22. Corbi D, Sunder S, Weinreich M, Skokotas A, Johnson ES, Winter E. 2014. Multisite phosphorylation of the Sum1 transcriptional repressor by S-phase kinases controls exit from meiotic prophase in yeast. *Mol. Cell. Biol.* 34:2249–63
23. Cormack BP, Ghori N, Falkow S. 1999. An adhesin of the yeast pathogen *Candida glabrata* mediating adherence to human epithelial cells. *Science* 285:578–82
24. Dang W, Steffen KK, Perry R, Dorsey JA, Johnson FB, et al. 2009. Histone H4 lysine 16 acetylation regulates cellular lifespan. *Nature* 459:802–7
25. De Las Penas A, Juarez-Cepeda J, Lopez-Fuentes E, Briones-Martin-Del-Campo M, Gutierrez-Escobedo G, Castano I. 2015. Local and regional chromatin silencing in *Candida glabrata*: consequences for adhesion and the response to stress. *FEMS Yeast Res.* 15:fov056

26. De Las Penas A, Pan SJ, Castano I, Alder J, Cregg R, Cormack BP. 2003. Virulence-related surface glycoproteins in the yeast pathogen *Candida glabrata* are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent transcriptional silencing. *Genes Dev*. 17:2245–58
27. Delgoshaie N, Tang X, Kanshin ED, Williams EC, Rudner AD, et al. 2014. Regulation of the histone deacetylase Hst3 by cyclin-dependent kinases and the ubiquitin ligase SCFCdc4. *J. Biol. Chem*. 289:13186–96
28. Devare MN, Kim YH, Jung J, Kang WK, Kwon KS, Kim JY. 2020. TORC1 signaling regulates cytoplasmic pH through Sir2 in yeast. *Aging Cell* 19:e13151
29. Domergue R, Castano I, De Las Penas A, Zupancic M, Lockatell V, et al. 2005. Nicotinic acid limitation regulates silencing of *Candida* adhesins during UTI. *Science* 308:866–70
30. Du J, Zhou Y, Su X, Yu JJ, Khan S, et al. 2011. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. *Science* 334:806–9
31. Durand-Dubief M, Sinha I, Fagerstrom-Billai F, Bonilla C, Wright A, et al. 2007. Specific functions for the fission yeast Sirtuins Hst2 and Hst4 in gene regulation and retrotransposon silencing. *EMBO J*. 26:2477–88
32. Edenberg ER, Vashisht AA, Topacio BR, Wohlschlegel JA, Toczyski DP. 2014. Hst3 is turned over by a replication stress-responsive SCF^{Cdc4} phospho-degron. *PNAS* 111:5962–67
33. Ellahi A, Rine J. 2016. Evolution and functional trajectory of Sir1 in gene silencing. *Mol. Cell. Biol*. 36:1164–79
34. Fabrizio P, Gattazzo C, Battistella L, Wei M, Cheng C, et al. 2005. Sir2 blocks extreme life-span extension. *Cell* 123:655–67
35. Fabrizio P, Longo VD. 2003. The chronological life span of *Saccharomyces cerevisiae*. *Aging Cell* 2:73–81
36. Fernandez J, Marroquin-Guzman M, Nandakumar R, Shijo S, Cornwell KM, et al. 2014. Plant defence suppression is mediated by a fungal sirtuin during rice infection by *Magnaporthe oryzae*. *Mol. Microbiol*. 94:70–88
37. Fine RD, Maqani N, Li M, Franck E, Smith JS. 2019. Depletion of limiting rDNA structural complexes triggers chromosomal instability and replicative aging of *Saccharomyces cerevisiae*. *Genetics* 212:75–91
38. Foss EJ, Gatbonton-Schwager T, Thiesen AH, Taylor E, Soriano R, et al. 2019. Sir2 suppresses transcription-mediated displacement of Mcm2–7 replicative helicases at the ribosomal DNA repeats. *PLOS Genet*. 15:e1008138
39. Freeman-Cook LL, Gomez EB, Spedale EJ, Marlett J, Forsburg SL, et al. 2005. Conserved locus-specific silencing functions of *Schizosaccharomyces pombe sir2*$^+$. *Genetics* 169:1243–60
40. Freeman-Cook LL, Sherman JM, Brachmann CB, Allshire RC, Boeke JD, Pillus L. 1999. The *Schizosaccharomyces pombe hst4*$^+$ gene is a *SIR2* homologue with silencing and centromeric functions. *Mol. Biol. Cell* 10:3171–86
41. Freire-Beneitez V, Gourlay S, Berman J, Buscaino A. 2016. Sir2 regulates stability of repetitive domains differentially in the human fungal pathogen *Candida albicans*. *Nucleic Acids Res*. 44:9166–79
42. Freire-Beneitez V, Price RJ, Tarrant D, Berman J, Buscaino A. 2016. *Candida albicans* repetitive elements display epigenetic diversity and plasticity. *Sci. Rep.* 6:22989
43. Froyd CA, Kapoor S, Dietrich F, Rusche LN. 2013. The deacetylase Sir2 from the yeast *Clavispora lusitaniae* lacks the evolutionarily conserved capacity to generate subtelomeric heterochromatin. *PLOS Genet*. 9:e1003935
44. Froyd CA, Rusche LN. 2011. The duplicated deacetylases Sir2 and Hst1 subfunctionalized by acquiring complementary inactivating mutations. *Mol. Cell. Biol.* 31:3351–65
45. Fu XH, Meng FL, Hu Y, Zhou JQ. 2008. *Candida albicans*, a distinctive fungal model for cellular aging study. *Aging Cell* 7:746–57
46. Ganley AR, Ide S, Saka K, Kobayashi T. 2009. The effect of replication initiation on gene amplification in the rDNA and its relationship to aging. *Mol. Cell* 35:683–93
47. Gerhart-Hines Z, Dominy JE Jr., Blattler SM, Jedrychowski MP, Banks AS, et al. 2011. The cAMP/PKA pathway rapidly activates SIRT1 to promote fatty acid oxidation independently of changes in NAD^+. *Mol. Cell* 44:851–63
48. Gottlieb S, Esposito RE. 1989. A new role for a yeast transcriptional silencer gene, *SIR2*, in regulation of recombination in ribosomal DNA. *Cell* 56:771–76

49. Ha CW, Huh WK. 2011. Rapamycin increases rDNA stability by enhancing association of Sir2 with rDNA in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 39:1336–50
50. Haldar D, Kamakaka RT. 2008. *Schizosaccharomyces pombe* Hst4 functions in DNA damage response by regulating histone H3 K56 acetylation. *Eukaryot. Cell* 7:800–13
51. Halme A, Bumgarner S, Styles C, Fink GR. 2004. Genetic and epigenetic regulation of the *FLO* gene family generates cell-surface variation in yeast. *Cell* 116:405–15
52. Hickman MA, Froyd CA, Rusche LN. 2011. Reinventing heterochromatin in budding yeasts: Sir2 and the origin recognition complex take center stage. *Eukaryot. Cell* 10:1183–92
53. Hickman MA, Rusche LN. 2009. The Sir2-Sum1 complex represses transcription using both promoter-specific and long-range mechanisms to regulate cell identity and sexual cycle in the yeast *Kluyveromyces lactis*. *PLOS Genet.* 5:e1000710
54. Honigberg SM. 2016. Similar environments but diverse fates: responses of budding yeast to nutrient deprivation. *Microb. Cell* 3:302–28
55. Hoppe GJ, Tanny JC, Rudner AD, Gerber SA, Danaie S, et al. 2002. Steps in assembly of silent chromatin in yeast: Sir3-independent binding of a Sir2/Sir4 complex to silencers and role for Sir2-dependent deacetylation. *Mol. Cell. Biol.* 22:4167–80
56. Hsu JY, Sun ZW, Li X, Reuben M, Tatchell K, et al. 2000. Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* 102:279–91
57. Humphrey KM, Zhu L, Hickman MA, Hasan S, Maria H, et al. 2020. Evolution of distinct responses to low NAD^+ stress by rewiring the Sir2 deacetylase network in yeasts. *Genetics* 214:855–68
58. Iida T, Kobayashi T. 2019. RNA polymerase I activators count and adjust ribosomal RNA gene copy number. *Mol. Cell* 73:645–54.e13
59. Imai S, Armstrong CM, Kaeberlein M, Guarente L. 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403:795–800
60. Itoh E, Odakura R, Oinuma KI, Shimizu M, Masuo S, Takaya N. 2017. Sirtuin E is a fungal global transcriptional regulator that determines the transition from the primary growth to the stationary phase. *J. Biol. Chem.* 292:11043–54
61. Itoh E, Shigemoto R, Oinuma KI, Shimizu M, Masuo S, Takaya N. 2017. Sirtuin A regulates secondary metabolite production by *Aspergillus nidulans*. *J. Gen. Appl. Microbiol.* 63:228–35
62. Jackson MD, Schmidt MT, Oppenheimer NJ, Denu JM. 2003. Mechanism of nicotinamide inhibition and transglycosidation by Sir2 histone/protein deacetylases. *J. Biol. Chem.* 278:50985–98
63. Jain N, Janning P, Neumann H. 2021. 14–3–3 protein Bmh1 triggers short-range compaction of mitotic chromosomes by recruiting sirtuin deacetylase Hst2. *J. Biol. Chem.* 296:100078
64. Kaeberlein M, Hu D, Kerr EO, Tsuchiya M, Westman EA, et al. 2005. Increased life span due to calorie restriction in respiratory-deficient yeast. *PLOS Genet.* 1:e69
65. Kaeberlein M, Kirkland KT, Fields S, Kennedy BK. 2005. Genes determining yeast replicative life span in a long-lived genetic background. *Mech. Ageing Dev.* 126:491–504
66. Kaeberlein M, McVey M, Guarente L. 1999. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev.* 13:2570–80
67. Kang WK, Kim YH, Kang HA, Kwon KS, Kim JY. 2015. Sir2 phosphorylation through cAMP-PKA and CK2 signaling inhibits the lifespan extension activity of Sir2 in yeast. *eLife* 4:e09709
68. Kapoor S, Zhu L, Froyd C, Liu T, Rusche LN. 2015. Regional centromeres in the yeast *Candida lusitaniae* lack pericentromeric heterochromatin. *PNAS* 112:12139–44
69. Keller NP. 2019. Fungal secondary metabolism: regulation, function and drug discovery. *Nat. Rev. Microbiol.* 17:167–80
70. Khodavandi A, Alizadeh F, Harmal NS, Sidik SM, Othman F, et al. 2011. Expression analysis of SIR2 and SAPs1–4 gene expression in *Candida albicans* treated with allicin compared to fluconazole. *Trop. Biomed.* 28:589–98
71. Kobayashi T, Ganley AR. 2005. Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats. *Science* 309:1581–84
72. Kobayashi T, Horiuchi T. 1996. A yeast gene product, Fob1 protein, required for both replication fork blocking and recombinational hotspot activities. *Genes Cells* 1:465–74

73. Kobayashi T, Horiuchi T, Tongaonkar P, Vu L, Nomura M. 2004. SIR2 regulates recombination between different rDNA repeats, but not recombination within individual rRNA genes in yeast. *Cell* 117:441–53
74. Konada L, Aricthota S, Vadla R, Haldar D. 2018. Fission yeast sirtuin Hst4 functions in preserving genomic integrity by regulating replisome component Mcl1. *Sci. Rep.* 8:8496
75. Krause DJ, Kominek J, Opulente DA, Shen XX, Zhou X, et al. 2018. Functional and evolutionary characterization of a secondary metabolite gene cluster in budding yeasts. *PNAS* 115:11030–35
76. Kruitwagen T, Denoth-Lippuner A, Wilkins BJ, Neumann H, Barral Y. 2015. Axial contraction and short-range compaction of chromatin synergistically promote mitotic chromosome condensation. *eLife* 4:e1039
77. Kumar S, Lombard DB. 2018. Functions of the sirtuin deacylase SIRT5 in normal physiology and pathobiology. *Crit. Rev. Biochem. Mol. Biol.* 53:311–34
78. Kwan EX, Foss EJ, Tsuchiyama S, Alvino GM, Kruglyak L, et al. 2013. A natural polymorphism in rDNA replication origins links origin activation with calorie restriction and lifespan. *PLOS Genet.* 9:e1003329
79. Landry J, Slama JT, Sternglanz R. 2000. Role of NAD^+ in the deacetylase activity of the SIR2-like proteins. *Biochem. Biophys. Res. Commun.* 278:685–90
80. Landry J, Sutton A, Tafrov ST, Heller RC, Stebbins J, et al. 2000. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *PNAS* 97:5807–11
81. Li C, Mueller JE, Bryk M. 2006. Sir2 represses endogenous polymerase II transcription units in the ribosomal DNA nontranscribed spacer. *Mol. Biol. Cell* 17:3848–59
82. Li G, Qi X, Sun G, Rocha RO, Segal LM, et al. 2020. Terminating rice innate immunity induction requires a network of antagonistic and redox-responsive E3 ubiquitin ligases targeting a fungal sirtuin. *New Phytol.* 226:523–40
83. Li M, Valsakumar V, Poorey K, Bekiranov S, Smith JS. 2013. Genome-wide analysis of functional sirtuin chromatin targets in yeast. *Genome Biol.* 14:R48
84. Li Y, Zhou Y, Wang F, Chen X, Wang C, et al. 2018. SIRT4 is the last puzzle of mitochondrial sirtuins. *Bioorg. Med. Chem.* 26:3861–65
85. Lim CS, Wong WF, Rosli R, Ng KP, Seow HF, Chong PP. 2009. 2-Dodecanol (decyl methyl carbinol) inhibits hyphal formation and SIR2 expression in *C. albicans*. *J. Basic Microbiol.* 49:579–83
86. Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, et al. 2002. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* 418:344–48
87. Lin YY, Lu JY, Zhang J, Walter W, Dang W, et al. 2009. Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. *Cell* 136:1073–84
88. Lindstrom DL, Leverich CK, Henderson KA, Gottschling DE. 2011. Replicative age induces mitotic recombination in the ribosomal RNA gene cluster of *Saccharomyces cerevisiae*. *PLOS Genet.* 7:e1002015
89. Liu B, Larsson L, Caballero A, Hao X, Oling D, et al. 2010. The polarisome is required for segregation and retrograde transport of protein aggregates. *Cell* 140:257–67
90. Low CF, Chong PP, Yong PV, Lim CS, Ahmad Z, Othman F. 2008. Inhibition of hyphae formation and SIR2 expression in *Candida albicans* treated with fresh *Allium sativum* (garlic) extract. *J. Appl. Microbiol.* 105:2169–77
91. Luo K, Vega-Palas MA, Grunstein M. 2002. Rap1-Sir4 binding independent of other Sir, yKu, or histone interactions initiates the assembly of telomeric heterochromatin in yeast. *Genes Dev.* 16:1528–39
92. Ma B, Pan SJ, Domergue R, Rigby T, Whiteway M, et al. 2009. High-affinity transporters for NAD^+ precursors in *Candida glabrata* are regulated by Hst1 and induced in response to niacin limitation. *Mol. Cell. Biol.* 29:4067–79
93. Ma B, Pan SJ, Zupancic ML, Cormack BP. 2007. Assimilation of NAD^+ precursors in *Candida glabrata*. *Mol. Microbiol.* 66:14–25
94. Maas NL, Miller KM, DeFazio LG, Toczyski DP. 2006. Cell cycle and checkpoint regulation of histone H3 K56 acetylation by Hst3 and Hst4. *Mol. Cell* 23:109–19
95. MacCabe AP, Riach MB, Unkles SE, Kinghorn JR. 1990. The *Aspergillus nidulans npeA* locus consists of three contiguous genes required for penicillin biosynthesis. *EMBO J.* 9:279–87
96. Marcet-Houben M, Gabaldon T. 2009. The tree versus the forest: the fungal tree of life and the topological diversity within the yeast phylome. *PLOS ONE* 4:e4357

97. Masumoto H, Hawke D, Kobayashi R, Verreault A. 2005. A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature* 436:294–98
98. McFaline-Figueroa JR, Vevea J, Swayne TC, Zhou C, Liu C, et al. 2011. Mitochondrial quality control during inheritance is associated with lifespan and mother-daughter age asymmetry in budding yeast. *Aging Cell* 10:885–95
99. Mead J, McCord R, Youngster L, Sharma M, Gartenberg MR, Vershon AK. 2007. Swapping the gene-specific and regional silencing specificities of the Hst1 and Sir2 histone deacetylases. *Mol. Cell. Biol.* 27:2466–75
100. Medvedik O, Lamming DW, Kim KD, Sinclair DA. 2007. MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in *Saccharomyces cerevisiae*. *PLOS Biol.* 5:e261
101. Michel AH, Kornmann B, Dubrana K, Shore D. 2005. Spontaneous rDNA copy number variation modulates Sir2 levels and epigenetic gene silencing. *Genes Dev.* 19:1199–210
102. Onishi M, Liou G-G, Buchberger JR, Walz T, Moazed D. 2007. Role of the conserved Sir3-BAH domain in nucleosome binding and silent chromatin assembly. *Mol. Cell* 28:1015–28
103. Orta-Zavalza E, Guerrero-Serrano G, Gutierrez-Escobedo G, Canas-Villamar I, Juarez-Cepeda J, et al. 2013. Local silencing controls the oxidative stress response and the multidrug resistance in *Candida glabrata*. *Mol. Microbiol.* 88:1135–48
104. Perez-Martin J, Uria JA, Johnson AD. 1999. Phenotypic switching in *Candida albicans* is controlled by a *SIR2* gene. *EMBO J.* 18:2580–92
105. Perrod S, Cockell MM, Laroche T, Renauld H, Ducrest AL, et al. 2001. A cytosolic NAD-dependent deacetylase, Hst2p, can modulate nucleolar and telomeric silencing in yeast. *EMBO J.* 20:197–209
106. Pierce M, Benjamin KR, Montano SP, Georgiadis MM, Winter E, Vershon AK. 2003. Sum1 and Ndt80 proteins compete for binding to middle sporulation element sequences that control meiotic gene expression. *Mol. Cell. Biol.* 23:4814–25
107. Price RJ, Weindling E, Berman J, Buscaino A. 2019. Chromatin profiling of the repetitive and nonrepetitive genomes of the human fungal pathogen *Candida albicans*. *mBio* 10:e01376-19
108. Roetzer A, Gabaldón T, Schüller C. 2011. From *Saccharomyces cerevisiae* to *Candida glabrata* in a few easy steps: important adaptations for an opportunistic pathogen. *FEMS Microbiol. Lett.* 314:1–9
109. Rupert CB, Heltzel JM, Taylor DJ, Rusche LN. 2016. Sporadic gene loss after duplication is associated with functional divergence of sirtuin deacetylases among *Candida* yeast species. *G3* 6:3297–305
110. Rusche LN, Kirchmaier AL, Rine J. 2002. Ordered nucleation and spreading of silenced chromatin in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 13:2207–22
111. Saka K, Ide S, Ganley AR, Kobayashi T. 2013. Cellular senescence in yeast is regulated by rDNA noncoding transcription. *Curr. Biol.* 23:1794–98
112. Sauve AA, Schramm VL. 2003. Sir2 regulation by nicotinamide results from switching between base exchange and deacetylation chemistry. *Biochemistry* 42:9249–56
113. Selles Vidal L, Kelly CL, Mordaka PM, Heap JT. 2018. Review of NAD(P)H-dependent oxidoreductases: properties, engineering and application. *Biochim. Biophys. Acta Proteins Proteom.* 1866:327–47
114. Shankaranarayana GD, Motamedi MR, Moazed D, Grewal SI. 2003. Sir2 regulates histone H3 lysine 9 methylation and heterochromatin assembly in fission yeast. *Curr. Biol.* 13:1240–46
115. Shen XX, Opulente DA, Kominek J, Zhou X, Steenwyk JL, et al. 2018. Tempo and mode of genome evolution in the budding yeast subphylum. *Cell* 175:1533–45.e20
116. Shigemoto R, Matsumoto T, Masuo S, Takaya N. 2018. 5-Methylmellein is a novel inhibitor of fungal sirtuin and modulates fungal secondary metabolite production. *J. Gen. Appl. Microbiol.* 64:240–47
117. Shimizu M, Masuo S, Fujita T, Doi Y, Kamimura Y, Takaya N. 2012. Hydrolase controls cellular NAD, sirtuin, and secondary metabolites. *Mol. Cell. Biol.* 32:3743–55
118. Sinclair DA, Guarente L. 1997. Extrachromosomal rDNA circles—a cause of aging in yeast. *Cell* 91:1033–42
119. Sipiczki M. 2006. *Metschnikowia* strains isolated from botrytized grapes antagonize fungal and bacterial growth by iron depletion. *Appl. Environ. Microbiol.* 72:6716–24
120. Smith DL Jr,. McClure JM, Matecic M, Smith JS. 2007. Calorie restriction extends the chronological lifespan of *Saccharomyces cerevisiae* independently of the Sirtuins. *Aging Cell* 6:649–62

121. Smith JS, Boeke JD. 1997. An unusual form of transcriptional silencing in yeast ribosomal DNA. *Genes Dev*. 11:241–54
122. Smith JS, Brachmann CB, Celic I, Kenna MA, Muhammad S, et al. 2000. A phylogenetically conserved NAD^+-dependent protein deacetylase activity in the Sir2 protein family. *PNAS* 97:6658–63
123. Stevenson JS, Liu H. 2011. Regulation of white and opaque cell-type formation in *Candida albicans* by Rtt109 and Hst3. *Mol. Microbiol.* 81:1078–91
124. Stumpferl SW, Brand SE, Jiang JC, Korona B, Tiwari A, et al. 2012. Natural genetic variation in yeast longevity. *Genome Res*. 22:1963–73
125. Tanner KG, Landry J, Sternglanz R, Denu JM. 2000. Silent information regulator 2 family of NAD-dependent histone/protein deacetylases generates a unique product, 1-*O*-acetyl-ADP-ribose. *PNAS* 97:14178–82
126. Thaminy S, Newcomb B, Kim J, Gatbonton T, Foss E, et al. 2007. Hst3 is regulated by Mec1-dependent proteolysis and controls the S phase checkpoint and sister chromatid cohesion by deacetylating histone H3 at lysine 56. *J. Biol. Chem*. 282:37805–14
127. Tsuchiya M, Dang N, Kerr EO, Hu D, Steffen KK, et al. 2006. Sirtuin-independent effects of nicotinamide on lifespan extension from calorie restriction in yeast. *Aging Cell* 5:505–14
128. Tudzynski B. 2014. Nitrogen regulation of fungal secondary metabolism in fungi. *Front. Microbiol.* 5:656
129. Turkel S, Ener B. 2009. Isolation and characterization of new *Metschnikowia pulcherrima* strains as producers of the antimicrobial pigment pulcherrimin. *Z. Naturforsch. C. J. Biosci.* 64:405–10
130. Vaquero A, Scher MB, Lee DH, Sutton A, Cheng HL, et al. 2006. SirT2 is a histone deacetylase with preference for histone H4 Lys 16 during mitosis. *Genes Dev*. 20:1256–61
131. Wilkins BJ, Rall NA, Ostwal Y, Kruitwagen T, Hiragami-Hamada K, et al. 2014. A cascade of histone modifications induces chromatin condensation in mitosis. *Science* 343:77–80
132. Wilson JM, Le VQ, Zimmerman C, Marmorstein R, Pillus L. 2006. Nuclear export modulates the cytoplasmic Sir2 homologue Hst2. *EMBO Rep*. 7:1247–51
133. Wilson RA, Talbot NJ. 2009. Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae*. *Nat. Rev. Microbiol.* 7:185–95
134. Winter E. 2012. The Sum1/Ndt80 transcriptional switch and commitment to meiosis in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 76:1–15
135. Wiren M, Silverstein RA, Sinha I, Walfridsson J, Lee HM, et al. 2005. Genomewide analysis of nucleosome density histone acetylation and HDAC function in fission yeast. *EMBO J.* 24:2906–18
136. Workman JJ, Chen H, Laribee RN. 2016. *Saccharomyces cerevisiae* TORC1 controls histone acetylation by signaling through the Sit4/PP6 phosphatase to regulate sirtuin deacetylase nuclear accumulation. *Genetics* 203:1733–46
137. Wurtele H, Tsao S, Lepine G, Mullick A, Tremblay J, et al. 2010. Modulation of histone H3 lysine 56 acetylation as an antifungal therapeutic strategy. *Nat. Med.* 16:774–80
138. Xie J, Pierce M, Gailus-Durner V, Wagner M, Winter E, Vershon AK. 1999. Sum1 and Hst1 repress middle sporulation-specific gene expression during mitosis in *Saccharomyces cerevisiae*. *EMBO J.* 18:6448–54
139. Zaganjor E, Yoon H, Spinelli JB, Nunn ER, Laurent G, et al. 2021. SIRT4 is an early regulator of branched-chain amino acid catabolism that promotes adipogenesis. *Cell Rep*. 36:109345
140. Zhao G, Rusche LN. 2021. Genetic analysis of sirtuin deacetylases in hyphal growth of *Candida albicans*. *mSphere* 6:e00053-21

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Errata

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