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Biochim Biophys Acta. Author manuscript; available in PMC 2013 June 19.

Published in final edited form as:
Biochim Biophys Acta. 2009 ; 1789(0): 443–450. doi:10.1016/j.bbagrm.2009.05.007.

Sin3:

Master Scaffold and Transcriptional Corepressor

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Abstract

Sin3 was isolated over two decades ago as a negative regulator of transcription in budding yeast. Subsequent research has established the protein as a master transcriptional scaffold and corepressor capable of transcriptional silencing via associated histone deacetylases (HDACs). The core Sin3-HDAC complex interacts with a wide variety of repressors and corepressors, providing flexibility and expanded specificity in modulating chromatin structure and transcription. As a result, the Sin3/HDAC complex is involved in an array of biological and cellular processes, including cell cycle progression, genomic stability, embryonic development, and homeostasis. Abnormal recruitment of this complex or alteration of its enzymatic activity has been implicated in neoplastic transformation.

Keywords

Sin3; Transcription; Deacetylation; Repression; Histones; Chromatin

1. Introduction

Sin3 was initially identified via genetic screen simultaneously by two groups in 1987-the MRC Laboratory of Molecular Biology (Cambridge, England) and the University of California, San Francisco (San Francisco, CA)-that were independently investigating the phenomena of mating type switching in budding yeast. Introduced as Sin3 (Swi-*in*dependent) and SDI1, respectively, these laboratories identified the protein as a negative regulator of the HO endonuclease responsible for catalyzing the mating type interconversion that is directly antagonized by several chromatin remodeling and activator SWI complexes [1, 2]. Subsequent studies rediscovered and reintroduced the protein under a number of different aliases (UME4, RPD1, GAM2, CPE-1, and SDS16, among others), primarily implicating the protein as negative transcriptional regulator, although roles as a positive transcriptional activator and enhancer of silencing have been described as well [3].

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Following over two decades of investigation, the functional portfolio of Sin3 currently encompasses numerous critical biological functions, including scaffolding of core histone deacetylase (HDAC) complexes, regulation of DNA and histone methylation, nucleosome remodeling, and N-acetylglucosamine transferase activity, among others. Interestingly, in spite of all these findings, however, the research on the structure and function of Sin3 and associated protein complexes remains an area of active investigation. Therefore, with the goal of informing the novice, as well as increasing the theoretical framework for future experiments in this area, in the current review, we describe the past and present developments in the characterization of Sin3, with particular emphasis on Sin3/HDAC complexes and their contribution to signaling and pathological processes.

2. Gene characterization and protein domains

The *SIN3* gene was first cloned in *S. cerevisiae* as a 5.8 kb DNA fragment containing a putative full length ORF encoding a large, acidic protein of 1538 a.a. (predicted 175kD) [4]. The protein's most prominent feature is its four paired amphipathic alpha-helix (PAH) motifs, which are imperfect repeats of roughly 100 residues each. Based on primary sequence analysis, each PAH motif was proposed to contain two α-helices; one exclusively hydrophilic, the other hydrophobic. Similar structures have been previously observed in the Myc family of transcriptional factors within the helix-loop-helix protein dimerization domains, even without possessing significant homology at the amino acid level, indicating Sin3's potential for a multitude of protein-protein interactions. Structural studies using NMR and X-ray diffraction methods, however, have revealed that, at least for the PAH1 and PAH2 domains, the primary structures function as independent domains that fold into a four-bundle structure [5]. Truncation assays suggest that the region between PAH3 and PAH4 is essential for the repressive activity of Sin3. However, the protein sequence is devoid of known DNA-binding motifs, indicative of recruitment to promoters via DNA-independent mechanisms [6].

Three isoforms of the Sin3 have been identified in mammalian cells, as products of two different genes, *SIN3A* and *SIN3B*. The defining difference between the two isoforms is that Sin3B possesses a shorter amino-acid tail prior to PAH1. Comparison with mouse mSin3 reveals striking similarity in the region of the PAH repeats [7, 8]. Two other highly conserved regions have also been identified, one in the region between PAH3-PAH4 (HDAC interaction domain, HID), and the other immediately following PAH4 (highly conserved region, HCR). Evidence also suggests the possibility of splicing variants for both mSin3A and mSin3B as a mouse Sin3B variant (mSin3B_{sf}) with an ORF of 293 a.a. has been reported [9-11].

3. Histone deacetylases: Sin3's enzymatic engine

A number of early studies identified RPD3, the yeast HDAC, and Sin3 as mutual partners with similar patterns of repression [12, 13]. Disruption of RPD3 or Sin3 produced phenotypes with similar features, including sensitivity to cycloheximide at 37°C, mating and sporulation defects, and enhanced silencing of genes ectopically positioned near telomeres [13-16]. Additionally, the *SIN3/RPD3* double mutants were found to possess defects similar to individual *SIN3* or *RPD3* mutants in *HO, IME2, INO1*, and *SPO13* gene transcription [12, 17]. Together, these studies strongly suggested that RPD3 and Sin3 functioned in the same genetic pathway. The mechanism underpinning the Sin3-RPD3 association in repression was further elucidated by the discovery of two highly related mammalian homologues of yeast, *RPD3-HD1* (HDAC1) and *mRPD3* (HDAC2). HDAC1 was isolated using the histone deacetylase inhibitor trapoxin [18], whereas HDAC2 was identified as a corepressor of transcription factor YY1 by a yeast two-hybrid screen [19].

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Meanwhile, the importance of histone acetylation in modulating chromatin structure and regulating transcription continued to gain momentum. Transcriptional coactivators, such as Gcn5, CBP/p300 and TAF_{II}250, were shown to possess histone acetylase activity [20-24]. Acetylation of core histones was thought to relax the nucleosome, relieving the repressive state to permit transcription factors and coactivators to gain access to recognition elements and initiate transcription. Conversely, corepressors were predicted to mediate transcriptional silencing via association with HDACs, which deacetylate core histones and stabilize the repressed state. Indeed, in 1997, these theories were bolstered with a series of papers describing the association of RPD3/HDACs and the role of histone acetylation in Sin3-mediation repression. Both HDAC1 and HDAC2 were demonstrated to be physically associated with mSin3 [25, 26]. Mad1, the first mSin3-interacting transcription factor identified, was found to form a complex with both Sin3 and HDAC2 with associated histone deacetylase activity. Detailed structural analyses have been performed to look at the interaction between Sin3 and Mad1. A complex of the Sin3 interaction domain (SID) of Mad1 bound to the PAH2 domain of Sin3a was evaluated by high-resolution NMR to determine that this interaction induces the collaborative conversion of relatively disordered structures into a highly ordered cluster of helices [27]. Mutation analyses revealed essential residues for maintaining an interaction between these two proteins, and subsequent studies demonstrated that insertion of PAH2-specific residues, and in particular Phe-7, into the PAH1 domain of mSin3b facilitated an interaction with the Mad-SID [28]. Although it was originally proposed that the minimal SID of Mad1 consisted of 13 residues, this domain was later extended to include 24 residues, upon findings that this extended SID significantly increased affinity for the PAH2 domain [29]. Additionally, HDAC1 and HDAC2 did not interact directly with Mad1 but were demonstrated to be recruited through their interaction with mSin3A via the highly conserved HID region though the defined mechanism for this association remains to be elucidated [25]. However, treatment of the cells with Trichostatin A (TSA), a histone deacetylase inhibitor, largely abolished Mad1-mediated repression, emphasizing the requirement of HDAC activity to achieve this repression.

Similar findings were also reported for RPD3. *In vivo* experiments demonstrated that UME6, a DNA-binding transcription factor involved in the regulation of meiosis-specific genes, specifically recruits Sin3 and RPD3 to its target promoters by direct interaction with Sin3. In Sin3 and RPD3 deletion stains, the UME6-mediated repression was greatly reduced or abolished, suggesting that both proteins are required to initiate repression [30]. Together, these studies established that Sin3-mediated silencing is achieved primarily through its associated histone deacetylase activities, a mechanism seemingly well conserved from yeast to human.

4. Identification of core Sin3/HDAC complex components

Further biochemical purification and characterization of the Sin3 complex revealed that, in addition to Sin3, HDAC1, and HDAC2, five other proteins comprise the core of the complex-RbAp4, RbAp7, SAP30, SAP18, and SDS3 [26, 31], as illustrated in Fig. 1. Supporting the validity of this data, yeast Sin3, similar to mSin3A, was also reported to be present in a large multi-protein complex [12]. Paralogs of members within this complex in both, *S. cerevisiae* and *S. pombe*, have been extensively mapped in a proteomic environment, termed “Chromatin Central”, as part of a study to combine protein quantification and bioinformatics to address the problem associated with isolating weak interactions, which are inevitably lost during purification steps, yet maintaining low background [32].

In the years following the elucidation of the core complex, a number of other associated proteins were uncovered, including SAP180 [33], RBP1 [33-35], BRMS1 [35, 36], SAP130

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[33], SAP25 [37], and ING1/2 [36, 38, 39]. The roles of these proteins remain largely unknown, however, it is theorized that some may function in specialized subsets of Sin3/ HDAC complexes. Evidence toward this theory has been illustrated in work with *S. cerevisiae*, which has demonstrated that Sin3/RPD3 exists as distinct, large (Rpd3L) and small (Rpd3S) variants [40]. While both complexes seem to share a core set of three subunits, including Rpd3, Sin3, and Ume1, they appear to have different functions. The large complex has been implicated in deacetylation at the promoter region [40-43], while the smaller complex appears to be responsible for deacetylation of coding regions, ultimately functioning to suppress spurious transcription by RNA polymerase II [42]. Given the multitude of proteins identified to interact with mSin3A, distinct Sin3/HDAC combinations are predicted to be associated with specific functions.

5. Deconstructing the Sin3 Complex

5a. The master scaffold

Currently, Sin3 is thought to be devoid of intrinsic DNA-binding abilities. The major mechanism of its ability to target chromatin is through direct interaction with DNA-binding transcription factors, such as with Mad1 or KLF repressors, or indirectly through adaptor proteins such as the nuclear hormone receptors via NCoR/SMRT, although an association between NCoR/SMRT is currently only speculative. Thus, Sin3 acts as a master scaffold to provide a platform for the assembly of numerous transcription factors and cofactors. Multiple domains within Sin3, such as its PAH repeats or HID region, serve as excellent protein-binding interfaces. Interestingly, almost all of the core components, including HDAC1/2, SAP30, SDS3/SAP45, SAP130, and SAP180, bind directly to the PAH3-HID region [26, 31, 33, 37, 44-47]. DNA-binding transcription factors are frequently observed to bind directly to the N-terminus PAH1 and PAH2 [48]. Although structural and sequence requirements of the PAH2 domain have been relatively well-defined, investigations on the requirements for binding to the PAH1 domain, which is closest in similarity, have recently initiated for interacting proteins, such as SAP25 [27]. The PAH4 and C-terminus HCR region are currently poorly characterized with very few identifiable protein interactions. Alien, a nuclear hormone corepressor, binds Sin3A in the HCR as well as HID regions [49]. Additionally, Sin3A is reported to recruit O-GlcNAc transferase (OGT) via PAH4 to promoters where they cooperatively repress transcription [50]. Sin3, therefore, appears to possess a modular nature with the central region dedicated to core complex assembly, the N-terminus reserved for chromatin targeting, and the C-terminus available for additional corepressor binding and intramolecular folding. However, a detailed analysis of additional Sin-3 binding proteins, particularly stoichiometric measurements of complex formation, is still necessary to shed further light on this subject.

The functional redundancy and specificity of the two isoforms of Sin3 is another important issue that remains largely unaddressed. Thus far, most of the characterization of Sin3 complex formation and protein-protein interactions has been limited to mSin3A. However, as the PAH and HID regions are fully preserved in mSin3B, it is expected that mSin3B may possess similar scaffolding capabilities. The degree of evolutionary conservation of both isoforms anticipates that at least some aspects of each isoform's function are unique and specialized, which would allow for greater flexibility during embryonic development and normal cellular function [3]. Studies to date have also indicated that these two isoforms target a similar subset of genes, although the differences in targets between the two have not been extensively analyzed [51]. Indeed, although numerous proteins, such as Mad1, KLF repressors, REST, and ESET [19], are observed to interact with both isoforms, others have been demonstrated to favor one or the other. SMRT and MeCP2 appear to bind to mSin3A [52, 53], while CIITA, the master regular of MHC II, mediates IFN-γ induced repression of collagen type I gene transcription via HDAC2/Sin3B rather than HDAC1/Sin3A [54] or

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E2F-Sin3B [55]. Sin3B has been identified as an essential and exclusive actor in promoting cell-cycle via the E2F-Rb pathway [56]. In fact, Sin3B deficient mice confirmed the role of mSin3B in cell cycle exit control and repression of E2F target genes *in vivo*, along with a role in the differentiation of erythrocytes and chondrocytes [57]. Mad1 binds both isoforms, however, LexA-Mad1 only copurifies with Sin3A in HeLa cells expressing both isoforms, suggesting the specificity of interaction may be cell-dependent or subject to signaling regulation [58, 59]. However, extremely scant information is currently available to explain the issue of isoform selectivity. Thus, careful dissection of Sin3A and Sin3B at the biochemical and functional levels is necessary to deduce the precise difference underlying each isoform.

The arrangement of PAH and HID regions within Sin3 is functionally relevant, as specific variants of both isoforms have been discovered that lack particular domains. One example is mouse $Sin3B_{SF}$ in which the first 327 amino acids are identical to those present in long-form mSin3B but are followed by a unique 19 residue stretch in the C-terminus, resulting in a truncated mSin3B with intact PAH1/2 domains but lacking the remainder of the C-terminus portion, including PAH3 and HID. Unsurprisingly, although $Sin3B_{SF}$ remains in the nucleus and is capable of repressing transcription in reporter assays, it fails to recruit HDAC and lacks anti-oncogenic activity in Myc/Ras-induced transformation of full-length mSin3B ($Sin3B_{LF}$) [44, 60]. The recruitment of NCoR, but not HDACs, by $Sin3B_{SF}$ has been suggested to provide a more attenuated and reversible type of regulation on the basal transcriptional apparatus, while $mSin3B_{LF}$ associates with both, presumably acting as a nucleosomal condenser to provide more effective multi-level gene repression. N-CoR, however, can recruit HDAC3 during mitosis, but whether Sin3 plays a role in this phenomenon remains elusive [61]. Detailed characterization of additional Sin3 variants and how they interact with other silencing complexes, such as N-CoR and different HDACs, may further shed light on these variant-specific regulatory mechanisms.

5b. HDAC1/HDAC2

These proteins belong to the family I of histone deacetylases and are thought to be primarily involved in transcriptional silencing in the nucleus in contrast to other HDAC families that can mediate cytoplasmic function. The HDAC, RPD3, and its mammalian orthologs, HDAC1/2, constitute the major catalytic subunits of Sin3/HDAC complexes in yeast and mammals, respectively. In yeast, studies of UME6-mediated repression suggest that RPD3 lacks specificity and is associated with general deacetylation of H3 and H4 preferentially at H4K5 and H4K12 [14, 62, 63]. In *Xenopus* oocytes, expression of the Mad1 SID fused to a Gal4 DNA-binding domain leads to targeted deacetylation of both H3 and H4 with substantially increased association of Sin3A/HDAC1 with chromatin [64]. Similarly, in an *in vitro* system using nucleosomal templates reconstituted with hyperacetylated histones or recombinant histones preaceylated with native SAGA or NuA4, selective targeting of mSin3/HDAC complexes resulted in deacetylation of both histones H3 and H4 [58]. In the same system, NCoR/SMRT complexes containing mainly HDAC3 resulted in deacetylation of only H3, suggesting that HDAC-containing complexes display distinct, intrinsic histone tail specificities [65].

Biochemically purified mSin3A/HDAC corepressor complexes are capable of repressing transcription from nucleosomal templates with acetylated H3 and/or H4 [59]. Histone deacetylase activity appears essential for repression of acetylated H3 templates but completely dispensable for H4 acetylated templates. Histone H3 acetylation may prevent a stable repressor-independent corepressor complex from targeting to immobilized nucleosome templates. As such, although both histone H3 and H4 are potential targets for deacetylation by the Sin3/HDAC1/2 complex, only the acetylation state of the N-terminus tail of histone H3 serves as a critical determinant for repressor-independent anchoring of the

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corepressor complex. These observations are instrumental in understanding the specificity and function of HDAC on chromatin as purified mSin3 complex contains both HDAC1 and HDAC2, but recombinant HDAC1/2 lacks deacetylase activity. Currently, it is unknown if H3/H4 deacetylation by mSin3A complexes is the result of the combined effect of both HDACs working in concert or if each HDAC is responsible for a particular histone. Additionally, both HDACs have been found to associate with corepressor complexes containing Mi-2/NURD [45, 66-68] and CoREST [69]. Mounting evidence also indicates that HDAC1/2 may function without additional biochemical partners. Corepressor complexes containing different HDACs with specificity toward different histone tails are predicted, thus future examination of substrate specificity and identification of *in vivo* targets will further shed light on this important aspect of gene silencing.

5c. RBBP4/7

The retinoblastoma binding proteins 4 and 7 (RBBP4/7, formerly RbAp48/46), which were first characterized in human cells as binding to the tumor suppressor, retinoblastoma (Rb), are believed to be essential for the silencing of cell cycle regulators [70, 71]. These proteins are the only components of the core Sin3/HDAC complex that do not interact directly with Sin3. Instead, the proteins are joined to the complex through the association of SAP30 or SDS3 and HDAC. As RBBP4/7 proteins are components of at least five chromatin-related complexes, all of which have histones as their substrates, it may be predicted that the RBBP subunits are the common point of interaction. Indeed, RBBP4 and 7 have been found to interact with histones H2A and H4, as well as the general histone octamer. However, these interactions were precluded when the histones were in association with DNA in the form of nucleosomes. An HDAC complex isolated from HeLa cell extracts was found to be capable of deacetylating core histone octamers but unable to deacetylate nucleosomal histones, supporting the theory that deacetylation of nucleosomal histones requires recruitment of HDAC complexes to DNA [72].

In vitro binding and reconstitution assays indicate that in the NURD complex, RBBP4/7 interact directly with HDAC1/2 to form a core complex required for HDAC1/2's enzymatic activity [26, 73], although in the Sin3 complex, RBBP4/7 has been demonstrated to have no effect on histone deacetylase activity [18]. Competition experiments have demonstrated that RBBP4/7 preferentially binds to hypoacetylated histone H3 over H4 [74], further supporting its role in targeting of the Sin3/HDAC complex to hypoacetylated promoters in the establishment of transcriptional repression [59]. This binding property of RBBP4/7 in Sin3A has also been suggested to be involved in the recognition and binding of hypoacetylated histone tails in pericentric heterochromatin where the Sin3 complex is present and actively involved in the maintenance of the hypoacetylated status [74, 75]. As the obligate members of the Sin3/NURD corepressor complex, RBBP4/7 proteins have a conserved function to assist in the physical stabilization of the contact between the complex and the nucleosome following their initial recruitment by DNA-binding transcriptional repressors.

5d. SDS3

SDS3 was originally identified in a screen for mutations that induced increased silencing of a crippled HMR silencer in a Rap1 mutant background. RPD3 and Sin3, then designated as SDS6 and SDS16, respectively, were simultaneously identified in the same screen [76]. SDS3 mutants displayed phenotypes very similar to those previously observed in Sin3 and RPD3 mutants [15]; most importantly that a mutation in SDS3 causing a marked reduction in Sin3-mediated repression. Finally, SDS3 and Sin3 were co-immunoprecipitated from cell extracts, indicating that both proteins interact with each other in the pathway [77].

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Further biochemical analysis has indicated that SDS3 is an integral subunit of the Sin3/RPD3 complex. In the absence of SDS3, Sin3 can be chromatographically separated from the RPD3 complex, resulting in a dramatic loss of its associated histone deacetylase activity [46]. Not surprisingly, the mammalian homologues of SDS3 (mSDS3) have been isolated as a Sin3-interacting protein in a yeast two-hybrid screen. mSDS3 interacts with the HID region of Sin3A/B but does not compete with HDAC1 for the HID site. The key functional properties of SDS3, such as chromatographic behavior, interaction with Sin3, and augmentation of HDAC activity, appear to be conserved from yeast to mammals [44, 46]. Germ-line or somatic deletion of mSDS3 generates a cell-lethal condition associated with rampant aneuploidy and general failure of cytokinesis. mSDS3-deficient cells fail to deacetylate pericentric heterochromatin histones, resulting in aberrant associations among heterologous chromosomes via centromic regions with consequent failure to segregate. Mutant mSDS3 molecules, incapable of Sin3-binding, fail to rescue mSDS3 null phenotypes. Thus, mSDS3 and its interaction with mSin3/HDAC components play a central role in initiating the cascade of pericentric heterochromatin-specific modifications necessary for the proper distribution of chromosomes during cell division in mammalian cells [78]. These studies indicate that SDS3 promotes the integrity of the Sin3 complex in addition to playing an important role in its catalytic activity. Furthermore, this data implicates SDS3 and Sin3 in the maintenance of chromosomal stability which disruption may be associated with lethality, developmental abnormalities and even diseases, such as cancer.

5e. SAP30

SAP30 is widely expressed in human tissues, particularly those of hematopoietic origin. Like Sin3, SAP30 is evolutionarily conserved from yeast to human with ample genetic and biochemical evidence to support that the protein exists in complex with the RPD3/HDAC complex in yeast and mammalian cells [25, 72]. In yeast, strains with the disruption of the SAP30 locus possess a similar phenotype to those with a mutation of either Sin3 or RPD3, suggesting that SAP30 may be required for the normal functioning of the Sin3/RPD3 complex. In cultured mammalian cells, SAP30 is directly associated with PAH3 of mSin3A as well as other components of the complex, including HDAC1 and RBBP4/7 [72]. Direct contact of SAP30 with multiple core components correlates with its roles in stabilizing the complex, a functional significance demonstrated by the fact that antibodies directed against SAP30 are capable of immunoprecipitating complexes active in deacetylating histone octamers, in particular histone H3.

In fact, accumulating evidence indicates that, in addition to mSin3A, SAP30 may serve as a bridging and stabilizing molecule between the Sin3A complex and other corepressors, such as RBP1, CIR, or DNA-binding transcription factors, such as YY1. Rbp1, a corepressor protein, recruits the mSin3/HDAC histone deacetylase complex to E2F-dependent promoters through direct interaction with SAP30 but not Sin3 or HDACs [34]. Direct interaction of SAP30 with DNA-binding transcription factors have also been reported. Papillomavirus binding factor, PBF (also known as Huntington's disease binding protein 2, HDBP2) is a nuclear-cytoplasmic shuttling transcription factor that binds to GC-rich sequence elements within upstream promoter regions of certain human papillomavirus (HPV) types and of the Huntington protein, respectively. PBF acts as a repressor of HPV transcription by recruitment of the Sin3/HDAC corepressor complex via direct interaction to SAP30 [79]. The same interaction and mechanism of repression is also predicted in two other closely related proteins, namely Glut4-EF/HDBP1 and GIG1.

Similarly, SAP30 is also reported to directly bind transcription factor YY1 and enhance YY1-mediated repression. Interestingly, only HDAC1, not Sin3 or RBBP4/7, was detected in the YY1/SAP30 complex, suggesting that SAP30 directly recruits HDAC1 instead of the Sin3/HDAC complex [19, 80]. The C-terminus region of SAP30 mediating YY1-binding is

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also required for Sin3-PAH3 binding which explains the mutually exclusive nature of SAP30 binding to mSin3A and YY1. Thus, in addition to being a subunit stabilizing the mSin3/HDAC complex, SAP30 may directly recruit the histone deacetylase to promoter DNA in a Sin3-independent manner; a notion supported by the direct interaction of SAP30/HDAC1 in mammalian cells and the fact that SAP30 can repress transcription in a *sin3*Δ mutant strain. A highly homologous protein, SAP30L, has been identified by virtue of its inducibility by TGFβ [81]. SAP30L is transcribed from a genomic locus independent from SAP30 (5q33.2 for SAP30L and 4q34.1 for SAP30). SAP30 and SAP30L share the biochemical and functional properties of mediating repression and interaction with Sin3 [82]. Interestingly, a functional nucleolar localization signal (NoLS) in both SAP30L and SAP30 has been identified which can target Sin3 to the nucleolus. Therefore, SAP30/SAP30L proteins may have a role in the targeting of the Sin3/HDAC corepressor complex into the nucleolus and participate in the regulation of rDNA transcription and/or rRNA processing [83]. However, strong evidence supporting the role of the Sin3 complex in the regulation of RNA polymerase promoters is lacking, thus pointing to a potentially fruitful area of investigation in the field of transcriptional regulation.

5f. SAP18

SAP18 is another polypeptide originally discovered by biochemical fractionation of human cell extracts and found to co-purify with the Sin3A/RPD3 HDAC complex in the enhancement of Sin3-mediated repression. This protein interacts directly with both mammalian Sin3 and HDAC1 and is suggested to have a potential role in stabilizing the HDAC1-Sin3 interaction and/or enhancing HDAC1 enzymatic activity [26]. Additionally, SAP18 has been found, mostly through yeast-two-hybrid screening, to interact with other proteins of various transcriptional regulatory circuits. For example, in *Drosophila melanogaster*, SAP18 directly interacts with GAGA and E(z) proteins, which are implicated in Trithorax and Polycomb-mediated regulation of homeotic genes, respectively [84, 85]. Therefore, it is possible that SAP18 is not an exclusive member of the Sin3 complex but, similarly to HDAC, can assist other molecular machinery in achieving gene silencing.

In *Drosophila melanogaster*, SAP18 interacts with Bicoid, transforming the protein from an activator into a repressor, permitting it to direct anterior patterning [86, 87]. In mammalian cells, SAP18 directly interacts and functionally cooperates with mouse homolog of Su(fu), a negative regulator of the Hedgehog signal-transduction pathway, to repress Gli-mediated transcription [88]. SAP18 also interacts with DPE2, the second largest subunit of mouse DNA polymerase epsilon, and may recruit HDAC to the replication fork to modify the chromatin structure, potentially for generating chromatin marks that are to be inherited epigenetically. More recently, chick hairy-enhancer-of-split protein, Hairy1, is suggested to repress target gene transcription through a direct interaction with the SAP18 protein [89]. In all these studies, SAP18 is proposed to be an adaptor protein that bridges interacting proteins and the Sin3/HDAC complex. Besides the aforementioned transcriptional repressors, SAP18 is also found to be part of a complex known as ASAP, which contains both an RNA-binding protein (RNPS1) and a caspase (Acinus) [90]. Along the same line, dSAP18 forms a complex with the *Drosophila melanogaster* homolog of Pinin (dPnn), a protein factor involved in mRNA splicing suggesting that it plays a role in RNA processing [91].

5g. Other components: RBP1, ING1/2, and SAP180

RBP1, a pRB pocket-binding chromodomain-containing protein, functions as a corepressor and bridging molecule between the RB family members and HDAC complexes [92]. RBP1 contains two repression domains (R1 and R2). While R1 functions independently of HDAC activity, it requires SUMOlation for the R2 domain to repress transcription in an HDAC-dependent manner [92, 93]. Further studies have indicated that the recruitment of HDAC

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activity by RBP1 is mediated through direct interaction with SAP30 of mSin3/HDAC complex, which accounts for at least 50% of RB-associated HDAC activity. Other bridging factors, including RBBP4/7, may contribute to the remaining activity. Thus, RBP1 allows recruitment of mSin3 HDAC complex to induce cell cycle arrest by repressing E2F dependent transcription and DNA replication origins [34].

ING1/2 was independently isolated as a SAP30-associated protein present in a specific subset of Sin3/HDAC complexes. The N-terminus of $p33^{ING1b}$ associates with the Sin3/HDAC1 complex through direct interaction with SAP30, which is the only subunit of the Sin3 complex that makes direct contact with p33. This interaction is required for the chromatin remodeling functions of p33ING1b [38]. A more recent study indicates that, like $p33^{ING1b}$, the human ING2 protein is also associated with similar components of mammalian Sin3/HDAC complexes, along with the RBP1 and its paralog, Rbp1-like protein, as well as Breast Metastasis Suppressor 1 (BRSM1) [93].

Rbp1-like protein (also reported as BCAA, RBP1-like 1, ARID4B, and SAP180) has previously been identified as a novel component of the Sin3/HDAC complex along with RBP1 and SAP130 [33]. The Rbp1-like protein is recently reported to possess similar biochemical and functional properties to RBP1, including serving as a stable component of the mSin3A/HDAC core complex and directly interacting with SAP30, to profoundly inhibit cell growth upon overexpression [33]. Although both RBP1 and $p33^{ING1b}$ (and likely ING2) directly bind SAP30, the simultaneous presence of BRSM1, INGs and RBP1 in the Sin3/HDAC-associated complex suggests that the bindings are cooperative rather than mutually exclusive and, in the case of BRMS1, the result of multiple points of contact.

In conclusion, Sin3's associated proteins not only provide structural stability within the Sin3/HDAC core complex, but also extend the interaction interface of the Sin3/HDAC complex to DNA-binding transcriptional repressors and other transcriptional regulatory proteins, allowing the complex to have extended flexibility and specificity.

6. Targeting and regulation of Sin3 complexes

Targeting of Sin3/HDACs complexes to chromatin is essential for the regulation of transcription. Sin3 and the other core components of the complex have no intrinsic DNA-binding activity and, as such, the major mechanism of gene-specific targeting of Sin3 corepressor complexes is through interaction with DNA-bound proteins. Following the discovery of the first Sin3-interacting transcriptional repressor, Mad1 [94], a surprisingly wide range of DNA-binding proteins have been found to interact with Sin3, making it, by far, the most widely studied corepressor complex for transcriptional repression. Most repressor/corepressor proteins interact with Sin3 via its multiple PAH motifs, in particular the PAH1-3 motifs in the N-terminus half. Although the PAH domains of Sin3 are significantly homologous to one another, especially between PAH1 and PAH2, each one can interact with a unique subset of proteins. The best-characterized interaction is the one between PAH2 and the Sin-3 Interaction Domain (SID), as described in SID-possessing proteins, KLFs and UME6 [95, 96]. The factors and corresponding interaction interfaces have been elucidated in an exhaustive fashion, as reported by Silverstein et. al [3].

The Sin3/HDAC corepressor complex can be recruited by a large number of DNA-binding transcription factors or corepressors, thereby requiring a precise and coordinated mechanism to achieve specific and timely regulation of transcription. For example, a recent report described coordinated recruitment of Sin3/E2F4 complexes and they mutually influence each other's binding position on a large subset of E2F4-regulated genes [51]. Additionally, signaling regulation and phosphorylation of DNA-binding proteins, such as AML1, has been shown to regulate transcription via disruption of repressor-corepressor interaction [97].

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Although poorly investigated, phosphorylation and/or other post-translational modifications are among the components of the corepressor complex that may be responsible for fine-tuning of regulation. For example, a recent study demonstrated that KLF11 represses the Smad7 promoter through binding to GC-rich promoter elements and subsequent recruitment of the mSin3a complex, terminating Smad7's autoinhibitory feedback loop and leading to an increase in Smad signaling. Activation of the Ras-Mek-Erk pathway, however, leads to the phosphorylation of KLF11, abrogating its ability to enhance Smad signaling [98]. Additionally, three distinct splicing variants of SAP30L have been identified, all of which differ in both localization and resultant repression efficiency, suggesting a possible method of Sin3A regulation based solely on associated proteins in addition to the integration of sequence-specific corepressors [99].

7. Physiological and pathological correlations

The power behind the Sin3 complex rests in the modular aspect of its composition. The addition or combination of associated corepressors and transcription factors into unique complexes enables unparalleled levels of precision in gene repression. Additionally, studies have demonstrated that the Sin3 complex is capable of incorporating additional catalytic units, including the Swi/Snf remodeling complex [100], O-GlcNAc transferase [19, 50], and histone H3-specific methyltransferase [19].

As a result, since its discovery, Sin3's repertoire, as summarized in Table 1, has expanded to included a wide variety of functions beyond cell cycle growth and proliferation, including DNA methylation (imprinting and X-chromosome inactivation), replication of centromeric chromatin, DNA damage repair, among others, even gene activation. A better understanding of the molecular mechanisms underlying complex formation and regulation is necessary to determine the exact role of the Sin3/HDAC complex in physiological and pathological cycles of these processes.

Clinical studies probing the relationship between alterations in Sin3 levels and corresponding disease has focused primarily on the Sin3 complex's associated corepressors. However, a recent study directly investigated decreased expression of Sin3A in the development of non-small cell lung cancer (NSCLC) via loss of heterozygosity analysis in a small cohort of 66 patients. The down-regulation of Sin3A mRNA in 61% of cases suggests that the decreased levels of Sin3A may lead to epigenetic deregulation of growth-related genes, resulting in tumorigenesis [101]. Lower levels of Sin3B have been confirmed in clear cell renal carcinoma [102]. Thus, the direct role of Sin3 in the disease state is just beginning to emerge.

8. Summary and outlook

Similar to other fields, recent advances in the study of Sin3 have answered a few very important questions and generated additional thoughts, which, if addressed, will further fill the gap in the existing knowledge. Within the scope of this review, we identify some future directions for research. For instance, both, defined biochemical and biophysical studies are needed to better understand the mechanisms and circumstances in which Sin3 functions as a scaffold. Surprisingly, for instance, a better mapping of the interaction of Sin3 and HDAC is needed. Similarly, how the interactions between these molecules are affected by signaling and how these proteins join Sin3a to perform its function represent an interesting niche for research. Furthermore, how many Sin3 complexes exist and in which context they work remain to be extensively addressed. For instance, we currently know that some proteins, which are part of the basic transcriptional machine, form complexes that are tissue-specific (tissue-specific TATA binding proteins). We predict that this Sin3-mediated cell-specific mechanism exists, though final proof of this phenomenon and as to what extent it works

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remains to be determined. Lastly, a plethora of structural studies are still needed to define, at the molecular level, how different proteins interact with each other within the complex to perform defined functions. Therefore, just studying the biochemistry and biophysics of the Sin3 molecules are very promising areas of future investigation.

Another important conceptual framework that is lacking in the field is whether Sin3 repression is independent, or it initiates a gene silencing process that is completed by other silencing machines. In fact, Sin3a has been unexpectedly linked with both short- and long-term repression, therefore, the mechanism underlying this phenomenon warrants these investigations.

Noteworthy, a significant area of cell biological interest is whether the three different Sin3 isoforms have redundant and/or different functions. For instance, the short Sin3B isoform could potentially work as a dominant negative since it contains the PAH1 and 2 domains but lacks the HDAC-recruiting module. On the other hand, however, it is possible that this isoform still represses by recruiting HDACs via other signaling complexes. In addition, carefully analysis of Sin3a and Sin3b isoforms predict a similar biochemical function as scaffolds, but they show different sites for posttranslational modifications which lead us to predict that signaling-induced regulation may differentially influence the function of these proteins. Although some of these studies are already underway, we are far from clarifying this area of knowledge and therefore, significant efforts must be devoted to better understand why higher eukaryotes have evolved to have three different isoforms of Sin3.

Furthermore, studies on Sin3 targets are currently underdeveloped, and in particular, those examining how either the sequence-specific transcription factors or the Sin3 complexes are modulated by distinct membrane-to-nucleus signaling to achieve their biological role. The results of these studies are important to build meaningful pathways for predicting potential novel functions of Sin3, which will advance our understanding of the cell biological function of this transcriptional scaffold.

Studies on Sin3 association with other histone deacetylase complexes also warrant further attention. During the last two decades, it has been clearly demonstrated that other silencing molecules interact with members of the Sin3 complex. However, the specific molecular details of these interactions and their cell biological consequences remain poorly understood. Therefore, addressing these questions will illuminate how these interactions exert a synergistic or antagonistic effect on gene expression, physiology and/or pathobiology of human diseases.

HDAC inhibitors have gained a significant interest due to their role as potential therapeutic tools to fight neoplastic diseases. However, we currently know that some of the HDAC-associated complexes function as tumor suppressors, while others as oncogenes. These concepts support the idea that current HDAC inhibitors, which rely on inhibiting the enzymatic activities of these enzymes, may bring about significant side-effects and give rise to drugs with poor therapeutic index. Thus, the discovery of a new generation of more specific HDAC inhibitors is needed and theoretically feasible if more specific knowledge is generated as to how HDAC, Sin3, and other associated signaling molecules form complexes. The major concept would be to target specific HDAC interactions, with other silencing machines that use them as their mechanism of action, to derive new drugs. For instance, one can envision that the interaction between HDAC and Sin3a can specifically be disrupted without interfering with the N-CoR-HDAC interaction, thus avoiding undesirable effects. Therefore, we predict that we are at the beginning of an explosion of knowledge in this important area of research with significant medical relevance.

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In summary, we here mention few, but extremely important new directions. We are excited with the possibilities that addressing these questions may bring as new knowledge. More importantly, these areas need to be explored to gain a better understanding of the cell and pathobiological contributions of Sin3, as well as the mechanisms underlying these functions. The discovery of Sin3 and its subsequent biochemical and mechanistic elucidation have already constituted a major advance in the field of transcriptional repression. The functional repertoire of Sin3 continues to expand at a rapid rate. The incredible flexibility afforded by the modular nature of the Sin3/HDAC complex provides Sin3 with unparalleled control in regulating gene transcription.

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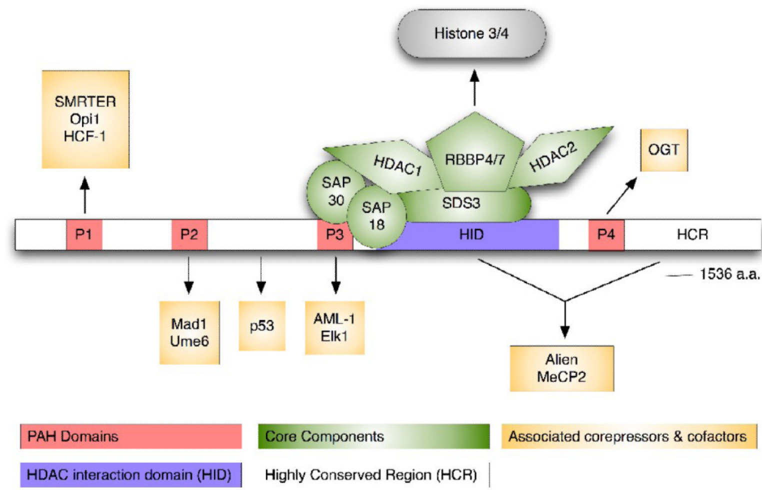

Figure 1.
The Sin3 protein is 1536 amino acids in length, possessing six highly conserved regions.

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Table 1

Summary of Sin3's repertoire of diverse activities

Activity	Function
DNA methylation	MeCP2 associates with Sin3A exclusively leading to deacetylation [93] and methylation of histone tails [94]
Gene activation	Hog1 recruits RPD3 to the promoters of osmoresponsive genes under conditions of stress [95]
Chromosome segregation	p33ING1 has been shown to serve as a bridge between the Sin3 complex and DNA methyltransferase (DNMT) and DNMT-associated proteins, localizing specifically in late S-phase [96]
rDNA silencing	rDNA silencing through association with nucleolar-remodeling complex (NoRC) [73]
DNA damage repair	Induction of double-strand breaks causes hypoacetylation of H4K16 in wild type but not Sin3 or RDP3 mutants in S. Cerevisiae [97]
Replication timing	Deletion of Sin3 or RDP3 causes early activation of late origins at internal chromosomal loci in *S. Cerevisiae* [98]