The Transcriptional Corepressor SIN3 Directly Regulates Genes Involved in Methionine Catabolism and Affects Histone Methylation, Linking Epigenetics and Metabolism*

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Chromatin modification and cellular metabolism are tightly connected. Chromatin modifiers regulate the expression of genes involved in metabolism and, in turn, the levels of metabolites. The generated metabolites are utilized by chromatin modifiers to affect epigenetic modification. The mechanism for this cross-talk, however, remains incompletely understood. The corepressor SIN3 controls histone acetylation through association with the histone deacetylase RPD3. The SIN3 complex is known to regulate genes involved in a number of metabolic processes. Here, we find that Drosophila SIN3 binds to the promoter region of genes involved in methionine catabolism and that this binding affects histone modification, which in turn influences gene expression. Specifically, we observe that reduced expression of SIN3 leads to an increase in S-adenosylmethionine (SAM), which is the major cellular donor of methyl groups for protein modification. Additionally, Sin3A knockdown results in an increase in global histone H3K4me3 levels. Furthermore, decreased H3K4me3 caused by knockdown of either SAM synthetase (Sam-S) or the histone methyltransferase Set1 is restored to near normal levels when SIN3 is also reduced. Taken together, these results indicate that knockdown of Sin3A directly alters the expression of methionine metabolic genes to increase SAM, which in turn leads to an increase in global H3K4me3. Our study reveals that SIN3 is an important epigenetic regulator directly connecting methionine metabolism and histone modification.

Cellular function relies on the ability of the cell to sense and respond to the environment. Cellular response is mediated in part by epigenetic and metabolomic information (1, 2). The expression of histone genes is under epigenetic control. Reduction of three histone modifiers, the H3K9 demethylase Jhdm2a, the H3K9/H3K56 deacetylase SIRT6, and the histone deacetylase HDAC1, leads to changes in metabolic gene transcription, as well as metabolites in mouse and rat models (3–5).

Because histone-modifying enzymes utilize key metabolites, these metabolites could then feedback and impact epigenetic modifications. Indeed, several groups have demonstrated that histone methylation can be altered through changes in metabolism. For example, histone methylation is regulated by threonine metabolism in mouse embryonic stem cells (6), by folate metabolism in yeast and human cells (7), and by methionine metabolism in yeast, fly, mouse, and human cells (7–10). Histone methylation and phosphorylation can also be modulated by changing glycolysis and serine metabolism in yeast (11). Although these studies collectively indicate that epigenetic control and metabolism are tightly connected, the mechanism for this cross-talk remains to be elucidated.

The SIN3 complex is one of the major histone-modifying complexes present in cells. SIN3 is a conserved transcriptional scaffold protein, which interacts with the histone deacetylase (HDAC)2 RPD3 and other associated proteins (12, 13). In Drosophila and mammals, a histone demethylase is also part of a SIN3/RPD3 HDAC complex (14). We previously reported a genetic interaction between Drosophila Sin3A and the genes encoding the histone demethylases KDM2 and dKDM5/LID (15, 16). These biochemical and genetic data suggest that the SIN3 complex may regulate histone methylation in addition to histone acetylation. Sin3A is essential in Drosophila and mammals (17–21). Deficiency of SIN3 leads to changes in expression of many genes involved in multiple biological processes, including cellular metabolism (16, 18, 22). SIN3 regulates genes involved in several metabolic pathways such as glycolysis, gluconeogenesis, and the citric acid cycle and additionally is associated with regulation of genes encoding proteins that process reactive oxygen species and glutathione (16, 18, 22–24). The mechanism of how SIN3 regulates cellular metabolism, however, is not fully understood.

Methionine, an essential amino acid, is converted to the major methyl donor S-adenosylmethionine (SAM) by SAM synthetase (SAM-S) (Fig. 1). SAM is then converted to S-adenosylhomocysteine (AHCY) to homocysteine, which is in turn
either converted to methionine through methionine synthase or to cystathionine by cystathionine-β-synthase (CBS). To date, according to FlyBase (25), Sam-S and Cbs are the only known Drosophila genes encoding SAM synthetase (26) and cystathionine-β-synthase, respectively. Ahcy13 is the major adenosylhomocysteinase gene (27). CG10623 encodes a putative methionine synthase. The metabolites involved in methionine metabolism are critical for multiple pathways and biological processes (28). For example, reduced methionine leads to decreased H3K4me3 in yeast and mammalian cells, at least in part by modulating SAM levels (7–9). Given that SIN3 may regulate histone methylation because of its biochemical or genetic association with the histone demethylases dKDM5/LID and KDM2 (15, 16, 29, 30), we wanted to examine further the relationship between SIN3 and methionine metabolism in Drosophila.

In this work, we focused on the mechanism through which SIN3 regulates cellular metabolism. We provided evidence that SIN3 binds to the promoters of methionine metabolic genes and affects H3K4me3 and H3K9ac levels at the promoter regions of these genes to control their expression. We observed increased levels of SAM and global H3K4me3 when SIN3 was reduced. Collectively, these results reveal that SIN3 regulates the expression of methionine metabolic genes through controlling histone modification levels at the promoters of these genes, which in turn regulates cellular SAM concentration and global H3K4me3.

Results

SIN3 Regulates Expression of Methionine Metabolic Genes and Histone Modifications at the Promoters of these Genes—Because SIN3 is a global transcriptional regulator (12, 13) and affects the expression of genes involved in several metabolic pathways such as glycolysis, gluconeogenesis, and the citric acid cycle (16, 18, 22, 23), we sought to determine whether SIN3 regulates the transcription of methionine metabolic genes. Analysis of our recently published RNA-Seq gene expression profiles of S2 and RNAi-mediated Sin3A knockdown cells (16) indicates that reduction of SIN3 alters the expression of Sam-S, Ahcy13, Cbs, and CG10623 (Fig. 2A). To verify the RNA-Seq data, we repeated the Sin3A knockdown experiment and analyzed mRNA levels by real time qRT-PCR. The knockdown of Sin3A was validated by Western blotting analysis (Fig. 2B). Consistent with the RNA-Seq data, transcription of these genes was significantly changed when SIN3 was reduced (Fig. 2C). These results demonstrate that SIN3 regulates the expression of methionine metabolic genes.

Next, we wanted to investigate how SIN3 affects the expression of these genes. Investigation of our recent ChIP-seq analysis (31), indicates that SIN3 binds to the promoters of Sam-S, Ahcy13, Cbs, and CG10623 (Fig. 3A). Additionally, changing the level of SIN3 alters global and gene specific histone acetylation, especially the H3K9ac mark (16, 29). Proteomic studies indicate that SIN3 copurifies with the H3K4me3 specific histone demethylase dKDM5/LID (29, 30), suggesting that the SIN3 complex may affect histone methylation. Based on these previous findings, we hypothesized that SIN3 directly controls H3K9ac and H3K4me3 levels at the promoters of methionine metabolic genes to regulate their expression. To test this hypothesis, we performed ChIP-qPCR analysis. IgG was used as a negative control.
a nonspecific control. Histone modification levels were normalized to histone H3 signal. Compared with IgG, strong enrichment of signals for tested histone antibodies was observed at all promoter regions sampled (data not shown). Knockdown of Sin3A led to an increase of H3K9ac and H3K4me3 at Sam-S, Ahcy13 and CG10623, whereas little to no change in H3K9ac was observed at Cbs (Fig. 3B). Consistent with published ChIP-seq analysis of H3K4me3 in S2 cells (32), we did not detect H3K4me3 at the promoter of Cbs in either the control or Sin3A knockdown condition (data not shown).

Given that H3K9ac and H3K4me3 are associated with active genes (33, 34), their levels at the tested genes are consistent with our gene expression data (Figs. 2, A and C, and 3B).

Because SIN3 is part of an HDAC complex, which contains both a histone deacetylase RPD3 and a histone demethylase dKDM5/LID (15, 16), we asked whether the increase of both H3K9ac and H3K4me3 at the promoters of methionine metabolism genes in Sin3A knockdown cells is due to instability of the complex and degradation of RPD3 and dKDM5/LID following knockdown of Sin3A. Our published RNA-Seq data indicate that the expression of rpd3 and lid is not changed when SIN3 is decreased (16). Reduction of SIN3 did not affect the protein level of RPD3. Whole cell extracts from RNAi-treated cells were subjected to Western blotting analysis using the indicated antibodies. β-Actin acted as the loading control. Protein size markers are indicated on the right.

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FIGURE 3. H3K9ac and H3K4me3 levels at the promoters of methionine metabolic genes are regulated by SIN3. A, SIN3 ChIP-seq signals at methionine metabolic genes. B, ChIP-qPCR analysis of H3K9ac and H3K4me3 levels at the promoters of methionine metabolic genes. The results are the average of three independent biological replicates. The error bars represent standard error of the mean. Statistically significant results comparing individual knockdown samples to the control are indicated on knockdown samples. GFP RNAi cells are the control cells. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

B. Reduction of SIN3 does not affect the protein level of RPD3. Whole cell extracts from RNAi-treated cells were subjected to Western blotting analysis using the indicated antibodies. β-Actin acted as the loading control. Protein size markers are indicated on the right.

C. A change in the cellular concentration of SAM has been demonstrated to result in altered histone methylation (7–9). To examine whether SIN3 affects global histone methylation, whole cell protein extracts from dsRNA-treated S2 cells were probed with antibodies specific for distinct histone methylation marks, as well as histone H4 as a loading control. Because SIN3 regulates gene specific H3K4me3 levels (Fig. 3B), we first tested this mark. Knockdown of Sin3A resulted in a small, but reproducible, increase in global H3K4me3 levels (Fig. 4, B and C). H3K4me3 is more sensitive to cellular SAM concentration rel-
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FIGURE 4. Levels of SAM and global H3K4me3 are regulated by SIN3. A, effects of SIN3 on the cellular concentration of the metabolites involved in methionine metabolism. B, whole cell extracts from GFP RNAi control and Sin3A knockdown S2 cells were subjected to Western blotting analysis using the indicated antibodies. Protein size markers are indicated on the right. C, Western blots as shown in B were repeated with protein extracts prepared from at least three independent cultures, and the results were quantified after normalization to histone H4. The error bars represent standard error of the mean. Statistically significant results comparing individual knockdown samples to the control are indicated on knockdown samples. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Discussion

In this study, we provide mechanistic insight into the relationship between an epigenetic regulator and metabolism. We first confirmed that SIN3 affects the expression of methionine metabolic genes. Next, we found that SIN3 influences histone acetylation and methylation at the promoters of these genes. Importantly, we observed that SIN3 regulates the levels of SAM and global histone methylation. Given that SIN3 is localized to methionine metabolic genes, these findings indicate that SIN3 directly regulates histone modifications at methionine metabolic genes to modulate their expression, which in turn impacts cellular SAM and global H3K4me3 levels. To our knowledge, this is the first demonstration of a regulatory role of SIN3 on methionine metabolism and consequently on global H3K4me3.

It has been reported that changes in the amount of histone modifier enzymes affect metabolism (3–5). In this report, we demonstrate that altering SIN3, the scaffold protein for assembly of one of the two major cellular histone deacetylase complexes, affects the expression of metabolic genes to impact metabolism. The data support a model in which changing a scaffold protein will alter the assembly and/or recruitment of the functional histone-modifying complex, which then impacts gene expression. Correlation analysis of modENCODE development RNA-Seq data provided by FlyBase (25) revealed that the expression of Sin3A was not obviously correlated with the expression of tested methionine metabolic genes during development (data not shown). We hypothesize that control by the complex will occur because of a change in cellular environmental status that will impact SIN3 complex binding or assembly rather than transcriptional changes to complex components. It will be informative to identify complex component gene specific recruitment under different conditions.

SIN3 and methionine metabolism are conserved from yeast to mammals. The data of this report using the Drosophila model system are consistent with the reports on histone modifiers regulating metabolism in yeast and mammals, which strongly suggests that this process is evolutionarily conserved across different species. In addition to histone modifications, in mammals, but not in Drosophila, DNA can also be modified by methylation. The possible role of SIN3 in the control of DNA methylation is largely unexplored. One report indicates that reduction of SIN3 in human netra-2 cells leads to decreased SIN3 binding at the promoter of the gene encoding glial fibrillary acidic protein (GFAP) and increased Gfap gene expression but no change to DNA methylation (38). More work will be necessary to fully examine the link between SIN3 and DNA methylation in those organisms in which DNA methylation is a critical epigenetic mark.
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Our work indicates that SIN3 impacts both global and gene specific H3K4me3. These results are consistent with the previous finding that reduction of both mammalian SIN3A and SIN3B leads to increased H3K4me3 at a specific group of genes in differentiated C2C12 myotubes (39). Given that H3K4me3 is associated with active genes (33, 34), the regulatory role of SIN3 on histone methylation may contribute to the mechanism of how SIN3 affects transcription, which in turn regulates biological processes. Previous studies have found that methionine metabolism is sufficient to determine histone methylation at least in part by modulating SAM levels (7–9). Our data indicate that SIN3 impacts H3K4me3 through affecting the expression of the genes encoding enzymes in this pathway, which ultimately controls the levels of key metabolites. Given that the H3K4me3 specific demethylase dKDM5/LID interacts with SIN3 (29, 30), it is possible that dKDM5/LID also contributes to the effect of SIN3 on H3K4me3, which is an interesting area for further research.

Experimental Procedures

Cell Culture—Drosophila Schneider cell line 2 (S2) cells were cultured at 27 °C in Schneider’s medium (1×) containing 1-glutamine (Life Technologies), 10% heat-inactivated fetal bovine serum (Invitrogen), and 50 mg/ml gentamycin.

dsRNA Production—The protocols for generation of constructs containing targeting sequences in pCRII-Topo vector and production of dsRNA are previously described (10, 40).

RNA Interference—The RNAi procedure is previously described (10, 40). Western blotting analysis and RT-PCR assays were routinely carried out for both single- and double-RNAi-treated cells to verify efficient knockdown of Sin3A and methionine metabolic genes, respectively.

Real Time Quantitative RT-PCR Assay (qRT-PCR)—The protocols for RNA extraction, cDNA preparation and qRT-PCR are previously described (10). Taf1 was used as a normalizer. The primers used for Taf1, Sam-S, Ahcy13, CG10623, and Cbs are previously described (10). Primers for Set1 were taken from a previously published report (37). Primers used for other genes are listed in Table 1. The gene expression changes are represented as the mean ± S.E. of the fold changes observed in Sin3A knockdown cells compared with GFP RNAi control cells. The qRT-PCR experiment utilized RNA isolated from three biological replicates for each cell type.

Metabolomics—Five biological replicates of RNAi-treated Drosophila S2 cells were harvested, flash frozen, and sent to Metabolon Inc. Sample preparation and metabolomic analysis were conducted at Metabolon Inc. as previously described (41). The extracted samples were split into equal parts for analysis with UPLC-MS/MS and GC/MS. Statistically significant differences were determined using one-way analysis of variance with post hoc contrasts (t tests).

Western Blotting Analysis—The Western blotting analysis protocol is previously described (10, 40). Primary antibodies included SIN3 (1:2000 (42)), α-tubulin (1:1000; Cell Signaling), H3K4me2 (1:5000; Millipore), H3K4me3 (1:2500; Active Motif), H3K9me2 (1:500; Millipore), and H4 (1:15,000; Abcam). Donkey anti-rabbit HRP-conjugated IgG (1:3000; GE Health-
TABLE 1

| Gene  | Primer orientation | Primer sequence (oriented 5’ to 3’) |
|-------|--------------------|-----------------------------------|
| trr   | Forward            | CAG TCG CGA GGT TAT CGG           |
| trx   | Forward            | TCC ATG CAG CGG GAA CAT           |
|       | Reverse            | CGG AAG TGC CGA AGT AAG           |
| ash1  | Forward            | GTC CCC AGA AGA GGC CAT           |
|       | Reverse            | CTC ATT GGC GAA TGC TAC           |

TABLE 2

| Gene  | Primer orientation | Primer sequence (oriented 5’ to 3’) |
|-------|--------------------|-----------------------------------|
| Sam-S | Forward            | CCA CAC TTC CAC CGT CTA CT        |
|       | Reverse            | CTA CTG TTC TCC AAG TGC AA        |
| Ac913 | Forward            | CAG AGC CCA GCT ACA AGG TC        |
|       | Reverse            | AAT AGA TGC AAT TCA CCC GC        |
| CG10623| Forward           | CGG AAA AGC TAG AGC AGT GA        |
|       | Reverse            | CGA TTT CAC CGA AGT TGT CT        |
| Cbs   | Forward            | TGC TCC TTC CTT TTT CAC TCT GA    |
|       | Reverse            | CGC QAA ATT CGG TGA QAT TA        |

care) was used as the secondary antibody. The antibody signals were detected using the clarity western ECL substrate (Bio-Rad) for H3K4me2 and H3K4me3 or ECL prime Western blotting detection system (GE Healthcare) for SIN3, α-tubulin, H3K9me2, and H4. A minimum of three biological replicates was performed.

Chromatin Immunoprecipitation and Real Time Quantitative PCR (ChIP-qPCR)—ChIP-qPCR procedure and antibodies are previously described (10, 16). Primers used for qPCR are listed in Table 2. Three biological replicates were performed.

Statistical Analyses—All significance values, except metabolomics experiment, were calculated by the unpaired two sample Student’s t test from GraphPad Software.

Author Contributions—M. L. and L. A. P. designed the study. M. L. performed experiments. M. L. and L. A. P. wrote the paper.

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