Fission Yeast Homologs of Human Histone H3 Lysine 4 Demethylase Regulate a Common Set of Genes with Diverse Functions

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Schizosaccharomyces pombe contains two proteins, SWIRM1 and SWIRM2, with close homology to human histone H3 lysine 4 demethylase. Both proteins contain the amino oxidase catalytic domain and a recently described DNA interaction SWIRM domain. Here we describe the biochemical isolation and the functional characterization of SWIRM1 and SWIRM2. Our results indicate that while SWIRM2 is an essential gene, cells lacking SWIRM1 are viable. We found that SWIRM1 and SWIRM2 are stably associated in a multiprotein complex, but intriguingly, unlike their human counterpart, S. pombe SWIRM complex contains neither a histone deacetylase nor any detectable demethylase activity. Genome-wide chromatin immunoprecipitation unexpectedly showed the absence of both SWIRM proteins from heterochromatic domains. Instead, consistent with biochemical analyses, SWIRM1 and SWIRM2 co-localize to a common set of target gene promoters whose functions are implicated in diverse processes including mitochondrial metabolism and transcriptional regulation. Importantly, we show that SWIRM1 is not only required for optimum transcription of its target genes but also display a global role in regulation of antisense transcription.

Histones, the building blocks of chromatin in eukaryotic cells, are subject to several posttranslational modifications including methylation, acetylation, phosphorylation, and ubiquitylation (1). These histone modifications play a central role in modulating chromatin structure and function (2, 3). Of particular interest in recent years, histone methylation is known to impact several cellular processes such as chromatin organization, genomic imprinting, and transcriptional regulation (4–6). This modification occurs on five lysine (K) residues of histone H3, H4, H2A, and H2B, and one lysine residue of histone H4 that can be methylated in three different modes, i.e. mono-, di-, or trimethylation. Generally, methylated H3K4, H3K36, and H3K79 residues are thought to be activation marks, whereas methylated H3K9, H3K27, and H4K20 residues represent repressive marks (4, 7). Arginine residues can also be methylated to generally lead to transcriptional activation (5).

Although other histone modifications such as acetylation, phosphorylation, and ubiquitylation have been known to be reversible, histone methylation was considered irreversible. However, recent discoveries of several histone demethylases that can reverse methylated lysine and arginine residues have altered our views of histone demethylation (8–13). PAD4/PADI4 was the first reported histone arginine demethylase that demethylinates monomethylarginine to produce citrulline (8, 9). BHC110/LSD1 has been identified as the first histone lysine demethylase that removes mono- or dimethyl-H3K4 by oxidation-based demethylation (13). More recently, a new family of histone demethylases was discovered to contain a JmjC domain capable of removing methyl groups on lysine residues by hydroxylation-based demethylation (10–12).

We and others (14–20) have purified BHC110 as a component of several multiprotein complexes. In addition, it has been demonstrated that BHC110 is recruited by REST (RE1-silencing transcription factor) to mediate silencing of neuronal-specific genes in non-neuronal cells (16). Importantly, it has been shown that nucleosomal demethylation by BHC110-containing complexes requires the SANT-domain-containing protein CoREST, reflecting an essential role for this protein in histone demethylation in vivo (21, 22). Strikingly, association of BHC110 with androgen receptor has been reported to change the enzyme specificity toward demethylating histone H3 lysine 9 (23).

In the present study, we isolated the two Schizosaccharomyces pombe homologs of BHC110, termed SWIRM1 and SWIRM2. Our in vivo characterization of these proteins suggests a role for SWIRM proteins in regulating transcription of diverse class of protein coding genes.

EXPERIMENTAL PROCEDURES

Affinity Purification of SWIRM1 and SWIRM2 Complexes—FLAG-SWIRM1 and FLAG-SWIRM2 complexes were purified...
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from whole cell extracts isolated from S. pombe strains expressing either FLAG-SWIRM1 or FLAG-SWIRM2 using anti-FLAG M2 affinity resin (Sigma). In brief, S. pombe cells were harvested and disrupted using glass beads. After centrifugation, anti-FLAG M2 affinity beads were incubated with whole cell extracts for 3–5 h, washed extensively with BC500 (20 mM Tris (pH 8.0), 500 mM KCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 10% glycerol) containing 0.1% Nonidet P-40, and eluted with FLAG peptide (Sigma). The FLAG affinity eluates were subjected to SDS-PAGE, followed by colloidal blue staining. The proteins were identified by liquid chromatography-tandem mass spectrometry.

Fission Yeast Strains—S. pombe strains carrying deletion either for SWIRM1 or SWIRM2 gene or expressing SWIRM1-green fluorescent protein (GFP) or SWIRM2-GFP were constructed using a PCR-based module method (24). SWIRM1 and SWIRM2 mutant strains were created by first replacing one of the two endogenous allele of a diploid strain with a kanamycin marker cassette followed by tetrad analysis of asygo-tic ascis to recover haploid cells containing the disrupted SWIRM allele.

Microscopy Analysis—To visualize the SWIRM1-GFP and SWIRM2-GFP proteins in live cells, freshly growing cells were stained with 1 μg/ml DAPI (4',6-diamidino-2-phenylindole) for 10 min, and subjected to microscopy.

ChIP-chip Analysis—ChIP-chip experiments were performed as described (25). Briefly, soluble chromatin fractions, prepared from strains expressing either FLAG-SWIRM1 or FLAG-SWIRM2, were incubated overnight with 20 μl of anti-FLAG M2 antibody agarose (Sigma), which was preblocked with 500 μg/ml bovine serum albumin. DNA recovered from the immunoprecipitated fractions and whole cell extracts were amplified by random priming PCR. Cy5-labeled ChIP DNA was combined with equal tated fractions and whole cell extracts were amplified by random priming PCR. Cy3-labeled whole cell extract DNA and hybridized onto an Agilent 44K 60-mer array. ChIP-chip data were

RESULTS

SWIRM2 but Not SWIRM1 Is an Essential Gene—Analysis of the S. pombe genome using human BHC110/LSD1 as queries revealed the presence of two uncharacterized open reading frames with close homology to the human histone H3 lysine 4 demethylase (Fig. 1A and supplemental Fig. 1). Both proteins contain the catalytic amine oxidase motif as well as the recently described SWIRM domain (26–28) and thus are termed SWIRM1 and SWIRM2 (Fig. 1A). Interestingly, while the S. pombe proteins contain an HMG domain in their C terminus, the human BHC110 does not contain an HMG domain. However, the HMG domain is represented in the BRAF35 protein (29), a subunit of human BHC110-containing complex required for transcriptional repression in vivo (17, 21, 30). Analysis of Saccharomyces cerevisiae genome did not reveal any protein with substantial homology to the S. pombe or human SWIRM-related proteins.

We next analyzed whether the SWIRM1 or SWIRM2 proteins fulfill essential functions in S. pombe. One of the two endogenous alleles was replaced with kan™ marker gene in diploid cells, and haploid cells were recovered following tetrad analysis of asygo-tic ascis (Fig. 1B). This analysis revealed that while SWIRM1 deletion results in decreased growth, SWIRM2 is an essential gene (Fig. 1B). Moreover, analysis of SWIRM1-deleted cells revealed aberrant protrusions and multinuclei phenotype associated with aberrant elongation (Fig. 1C), which is similar to phenotypes displayed by cells carrying mutations in components of Clr6 HDAC complex (31). Finally, microscopic examination of cells expressing SWIRM1 or SWIRM2 proteins fused to GFP confirmed the nuclear localization of both proteins (Fig. 1D).

Isolation of Multiprotein Complexes Containing SWIRM1 and SWIRM2—To isolate SWIRM1- and SWIRM2-containing complexes, we constructed strains expressing FLAG-SWIRM1 and FLAG-SWIRM2. FLAG-SWIRM1 and FLAG-SWIRM2 were isolated using affinity chromatography, and the affinity eluate was subjected to SDS-PAGE followed by Colloidal Blue staining. Mass spectrometric analysis indicated that SWIRM1 and SWIRM2 proteins are associated together as FLAG-SWIRM1 affinity eluate contains SWIRM2 and vice versa (Fig. 2). Moreover, we found an RNA recognition motif-containing protein, SPBPJ758.01, as a common subunit associated with both SWIRM1 and SWIRM2 affinity eluates. Additionally, two related plant homeodomain-containing proteins (SPAC 30D11.08C and SPCC 4G3.07c) were identified to interact with SWIRM2 and SWIRM1, respectively. Interestingly, the SWIRM2 affinity eluate also contains sequences corresponding to the chromatin remodeling protein Hrp1 (32). These results indicate that unlike its human homolog BHC110, S. pombe SWIRM proteins do not form a stable complex with histone deacetylases. Moreover, our data suggest that SWIRM1 is present in at least one kind of complex without SWIRM2 and vice versa. Finally, while human BHC110-containing complexes
Twenty independent asci derived from (SPAC23E2.02) genes is registered at the domain proteins, SWIRM1 and SWIRM2. The sequence information of markers are depicted on the identity. They both contain a SWIRM (Swi3, Rsc8, and Moira) (NOVEMBER 24, 2006•

by mass spectrometry is shown to the SDS-PAGE using colloidal blue staining. The identity of polypeptide analyzed “Experimental Procedures.” The FLAG affinity eluate was analyzed following FLAG-SWIRM1 and FLAG-SWIRM2 proteins were isolated as described under Isolation of SWIRM1- and SWIRM2-containing complexes.

FIGURE 1. Deletion analysis of SWIRM domain proteins in S. pombe. A, a schematic representation of two SWIRM domain proteins, SWIRM1 and SWIRM2. The sequence information of SWIRM1 (SPBC146.09c) and SWIRM2 (SPAC23E2.02) genes is registered at the S. pombe gene data base web site. SWIRM1 and SWIRM2 proteins share 29% identity. They both contain a SWIRM (Swi3, Bsc8, and Moira) (red), an amine oxidase (yellow), and a HMG (high mobility group) (green) domains. B, SWIRM2, but not SWIRM1, is an essential gene. Deletion of SWIRM1 and SWIRM2 genes were carried out by replacing one of the two endogenous alleles with kan marker gene in diploid cells. Twenty independent asci derived from SWIRM1::kan or SWIRM2::kan diploid cells were subjected to tetrad analysis. C, phenotype of SWIRM1Δ. Deletion of SWIRM1 gene causes elongation, abnormal protrusions (budding phenotype), and multinuclei phenotype. Wild-type and SWIRM1Δ cells were analyzed by light microscopy (differential interference contrast (DIC), left panels) and DAPI staining (right panels). D, nuclear localization of SWIRM1 and SWIRM2 proteins. Localization of SWIRM1-GFP and SWIRM2-GFP signals in live cells were visualized by detecting the GFP fluorescence (left panels). DAPI signals indicate nuclei (right panels).

FIGURE 2. Isolation of SWIRM1- and SWIRM2-containing complexes. FLAG-SWIRM1 and FLAG-SWIRM2 proteins were isolated as described under “Experimental Procedures.” The FLAG affinity eluate was analyzed following SDS-PAGE using colloidal blue staining. The identity of polypeptide analyzed by mass spectrometry is shown to the right of the figure. Molecular weight markers are depicted on the left.

Pombe SWIRM Proteins display histone H3 lysine 4 demethylase activity, repeated analysis of S. pombe SWIRM1/2-containing complexes failed to detect any histone demethylase activity (data not shown and supplemental Fig. 2). This represents analysis of SWIRM complexes for demethylation of dimethyl-histone H3 at lysines 4, 9, 27, 36, and 79. Additionally, recombinant SWIRM1 protein produced in bacteria failed to demethylate dimethyl-histone H3 at lysines 4, 9, and 36 (data not shown).

SWIRM1 and SWIRM2 Delineate the 5′ End of Genes and Co-localize to the Same Chromatin Regions—To gain insight into genomic localization of SWIRM1 and SWIRM2 proteins we performed genome-wide chromatin immunoprecipitation or ChIP-chip analysis across the three S. pombe chromosomes. This analysis revealed a remarkably similar co-localization pattern of SWIRM1 and SWIRM2 proteins at nearly all genomic loci, consistent with the presence of these two proteins in a same multiprotein complex (Fig. 3, A–D). Indeed, analysis of 175 loci that displayed high enrichment (>2-fold) for either SWIRM1 or SWIRM2 localization indicated that a majority of loci (94) are occupied by both SWIRM1 and SWIRM2 (Fig. 3 and supplemental Fig. 2). The 81 loci having little enrichment (<2-fold) for one of the SWIRM proteins tend also to have low enrichment for the other protein, suggesting that these loci are probably occupied by both proteins as well. Moreover, a detailed analysis of promoter regions for SWIRM1 and SWIRM2 occupancy revealed their co-localization near the start site of transcription, consistent with a role for these proteins in transcriptional regulation (Fig. 3B and supplemental Fig. 2).

Functional analysis of genes associated with SWIRM1 and SWIRM2 revealed a wide spectrum of cellular functions including mitochondrial metabolism, translation, intracellular protein transport, and transcriptional regulation (supplemental Table 1). Taken together, these results indicate that SWIRM1 and SWIRM2 proteins regulate a common set of genes involved in many biological pathways, which thus could explain the essential requirement of SWIRM2 for the viability of S. pombe.

Localization of the SWIRM1 and SWIRM2 Proteins Does Not Generally Coincide with Peaks of Histone H3 Lysine 4 Dimethylation—Since the human homolog of SWIRM1 and SWIRM2 protein, BHC110, is a histone H3 K4 demethylase that specifically removes dimethylation on lysines (13, 21, 22), we asked whether the SWIRM1 and SWIRM2 proteins display a similar distribution as that of dimethyl-H3K4. Previous genome-wide analysis revealed broad but varied distributions of dimethyl-H3K4 across S. pombe euchromatic regions, with a
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A

Chromosome I

5.7 Mb

tel1L cen1 tel1R

Chromosome II

4.6 Mb

tel2L cen2 mat tel2R

Chromosome III

2.5 Mb

tel3L rDNA cen3 rDNA tel3R

ChIP relative enrichment

SPAC630.15 cut6 ctf212

SPAC3409.06c ypl2

SPAC300.15

ChIP relative enrichment

SPAC630.15 cut6 ctf212

SPAC3409.06c ypl2

SPAC300.15

ChIP relative enrichment

SPAC630.15 cut6 ctf212

SPAC3409.06c ypl2

SPAC300.15

B

ChIP relative enrichment

cut6 SPAC56E4.05 mei1 act1 mok12

LTR SPCC1906.05

C

ChIP

act1
dg

WCE

D

Swim1 11 94 70 Swim2
higher enrichment of dimethyl-H3K4 toward the 5’ end of genes (25). However, overlaid distribution maps between those of the SWIRM proteins and dimethyl-H3K4 showed that SWIRM binding peaks do not generally overlap with dimethyl-H3K4 peaks (supplemental Fig. 2). However, for some genes a partial overlap is observed (supplemental Fig. 2).

**SWIRM1 Is Required for Optimal Transcription of Its Target Genes**—To assess the role of the SWIRM proteins in transcriptional regulation we compared the transcript levels for SWIRM1 target genes in the wild type cells and SWIRM1 deletion strains. Remarkably, absence of SWIRM1 results in a specific and profound decrease in transcriptional levels of the SWIRM1 target genes (Fig. 4, A and B). While deletion of SWIRM1 results in a specific decrease in transcription of sense strand, we observed a general increase in the levels of antisense transcripts in cells lacking SWIRM1. This effect was not specific to genes targeted by SWIRM1 and was also observed in non-SWIRM1 targets (Fig. 4A). Taken together, these results point to a specific role for SWIRM1 in expression of its target genes and a global role for this protein in suppression of antisense transcripts.

In this study, we report the characterization of two *S. pombe* SWIRM domain-containing proteins, SWIRM1 and SWIRM2, with close homology to histone H3 lysine 4 demethylase, BHC110. Interestingly, unlike the human protein the *S. pombe* proteins are not in a stable association with histone deacetylase subunits, and furthermore, we were not able to detect a demethylase activity toward either histones or nucleosomes. However, the isolation of native complexes containing SWIRM1 and SWIRM2 proteins indicated that the proteins are in a stable complex, pointing to a similar function for both proteins. This contention is further verified as our analysis of their chromatin occupancy revealed their association with a similar set of genes.

**DISCUSSION**

Our *in vivo* analysis indicated that while SWIRM2 is an essential gene, deletion of SWIRM1 leads to growth inhibition. Although both proteins seem to associate with a similar set of polypeptides, affinity purification of SWIRM2 led to the identification of HRP1 as a SWIRM2-associated protein. It is possible that SWIRM2 forms a distinct complex with HRP1 that does not contain SWIRM1 and that this complex may perform essential functions, which are abrogated upon SWIRM2 deletion. Moreover, we find additional subunits that contain plant homeodomain reminiscent of the BHC80 subunit in human complexes (17, 21, 30, 33). Of notable exception is the absence of SWIRM-associated subunits containing the SANT domain. The SANT domain-containing subunit CoREST is a critical component of enzymatic activities embedded in human BHC110-containing complexes (21).

Interestingly, genome-wide ChIP analysis of SWIRM1 and SWIRM2 proteins did not identify either centromeric or telomeric regions as sites where there is an enrichment of SWIRM proteins. These results are consistent with the notion that the SWIRM proteins may not play a role in heterochromatin-related gene silencing. Indeed, consistent with this contention, we find SWIRM proteins predominantly at transcription start sites, reflecting a role for these proteins in regulation of transcription.

**FIGURE 4.** SWIRM1 is required for the local activation of sense transcription and the global repression of antisense transcription. A, total RNAs were purified from wild-type (WT) and SWIRM1Δ strains. The levels of sense and antisense transcripts were examined by strand-specific RT-PCR for three SWIRM target genes (top panel) and two non-SWIRM target genes (bottom panel). Panels on right (−RT) show the absence of DNA contamination in the RNA samples. B, the quality of the RNA samples used in A and the relative quantity of RNA between the two strains are shown on an agarose gel.
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Moreover, in the absence of SWIRM1 we have found a profound decrease in transcription of SWIRM1 target genes, pointing to a role for SWIRM1 in regulation of transcription. Surprisingly, lack of SWIRM1 led to a strong increase in the level of the antisense transcripts corresponding to all genes examined. This effect of SWIRM1 deletion was not specific to SWIRM1 target genes and may result from the requirement of SWIRM1 for the transcription of a suppressor of antisense transcripts. Future experiments examining the specificity of targets of SWIRM1 in antisense transcription may provide further insight into the mechanism by which these transcripts are produced.

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