Histone H3 K36 Methylation Is Associated with Transcription Elongation in Schizosaccharomyces pombe

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Set2 methylation of histone H3 at lysine 36 (K36) has recently been shown to be associated with RNA polymerase II (Pol II) elongation in Saccharomyces cerevisiae. However, whether this modification is conserved and associated with transcription elongation in other organisms is not known. Here we report the identification and characterization of the Set2 ortholog responsible for K36 methylation in the fission yeast Schizosaccharomyces pombe. We find that similar to the budding yeast enzyme, S. pombe Set2 is also a robust nucleosome-selective H3 methyltransferase that is specific for K36. Deletion of the S. pombe set2+ gene results in complete abolishment of K36 methylation as well as a slow-growth phenotype on plates containing synthetic medium. These results indicate that Set2 is the sole enzyme responsible for this modification in fission yeast and is important for cell growth under stressed conditions. Using the chromatin immunoprecipitation assay, we demonstrate that K36 methylation in S. pombe is associated with the transcribed regions of Pol II-regulated genes and is devoid in regions that are not transcribed by Pol II. Consistent with a role for Set2 in transcription elongation, we find that S. pombe Set2 associates with the hyperphosphorylated form of Pol II and can fully rescue K36 methylation and Pol II interaction in budding yeast cells deleted for Set2. These results, along with our finding that K36 methylation is highly conserved among eukaryotes, imply a conserved role for this modification in the transcription elongation process.

Covalent histone modifications represent a major mechanism by which cells regulate the structure and function of chromatin. A number of different posttranslational modifications are known to occur on histones, including acetylation, methylation, phosphorylation, ubiquitylation, and, more recently, sumoylation (7, 17, 36, 41). While the majority of these modifications are restricted to the flexible N- and C-terminal tail domains of these proteins, a significant number of these modifications have been identified in their highly structured globular domains (11, 53). The function of these modifications are not well understood, but it is becoming increasingly clear that they coordinate their effects in the form of a histone code to regulate the complex and diverse activities associated with DNA in chromatin (21, 46, 50).

A large body of work now shows that histone methylation plays a key role in the regulation of chromatin structure and function. In particular, studies show that the methylation of lysine and/or arginine residues regulates diverse cellular functions such as transcriptional repression and activation, heterochromatin formation, X inactivation, and polycomb-mediated gene silencing (10, 14, 19, 23, 26, 54). More recently, studies have revealed an unexpected role for histone methylation in the process of transcription elongation by RNA polymerase II (Pol II). In the budding yeast Saccharomyces cerevisiae, the histone methyltransferases Set1 and Set2, which catalyze H3 lysine 4 (K4) and lysine 36 (K36) methylation, respectively, have been found to be associated with the elongation-competent form of Pol II (13, 15). While Set1 association is dependent on the Kin28 kinase, which phosphorylates the serine 5 (Ser5) position of the C-terminal domain (CTD) of Pol II (13, 15, 24, 31), Set2 association and methylation is dependent on Ctk1, which phosphorylates the serine 2 position of the CTD (25, 27, 28, 39, 52). While the precise function of these enzyme associations with Pol II is still unclear, it is believed that K4 and K36 methylation function in the elongation process at different stages of the transcription elongation cycle (42, 43).

To date, the association of Set2 with elongating Pol II has only been demonstrated in S. cerevisiae. Whether this enzyme has a conserved role and associates with Pol II in other organisms is not known. In the fission yeast Schizosaccharomyces pombe, Set1-mediated H3 K4 methylation is preferentially enriched at the euchromatic loci, in particular at the regions containing open reading frames (33, 34). Moreover, a potential ortholog of S. cerevisiae Set2 has been identified in S. pombe (34). In this report, we characterize the fission yeast S. pombe Set2 (SpSet2) and find that this enzyme is a robust K36 methyltransferase that mediates nucleosome-selective methylation. Similar to what is found in budding yeast, K36 methylation in S. pombe is restricted to the coding region of active genes, and we show that the SpSet2 enzyme interacts with Pol II and restores K36 methylation in S. cerevisiae when the endogenous SET2 gene is deleted. These studies, and the fact that K36 methylation is conserved across eukaryotes, suggest a highly conserved role for K36 methylation in transcription.
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MATERIALS AND METHODS

Yeast strains. The S. pombe yeast strains used in these studies were SP1173 (wild type; h- leu1-32 his2 ura4 ade6-216) and SPK549 (set2Δ h- leu1-32 his2 ura4 set2Δ kanMX6 cen1::ura4 ade6-210). For the growth assays, S. pombe strains SPK131 (wild type; h- leu1-32 his2 ura4 Rint2::ura4 ade6-216) and SPK612 (set2Δ h- leu1-32 his2 ura4 Rint2::ura4 set2Δ kanMX6 ade6-210) were used. The set2Δ strain was constructed by a PCR-based method using a kanMX6 module to replace the S. pombe set2′ gene as described (5). Deletion was confirmed by PCR and Southern blot analysis. SP1173 was used to set a genomic 3XFlag epitope tag in S. pombe strains as described (17). Using the SPSet2-Flag expression plasmid, SPK655 (h- leu1-32 ura4 set2Δ 2FLAG-cen1::ura4 ade6-210), S. cerevisiae wild-type and set2Δ strains in the BY4742 background were obtained from Research Genetics.

Preparation of histones. Histones were prepared as previously described (47). Briefly, nuclei were isolated by detergent lysis and low-speed centrifugation from 293T cells grown at 37°C in Dulbecco’s modified Eagle’s medium containing 5% fetal bovine serum. Histones were extracted from nuclei by either DNase I or acid extraction. Wild-type Tetrahymena thermophila was grown in enriched 1% fetal bovine serum. Histones were prepared from budding yeast, Tetrahymena thermophila, isolated histones from budding yeast, and calf thymus histones. Briefly, nuclei were isolated by detergent lysis and low-speed centrifugation from isolated nuclei as described (47).

Sequence analysis (Set2 homology search). Database searches and protein sequence identities were performed with BLAST (3) and PSI-BLAST (4). Sequences were aligned and the phylogeny tree was calculated using the neighbor-joining method in the program Clustal X (20). The resulting dendrogram was displayed using the tree drawing program NPlot (35).

Cloning of S. pombe Set2 and generation of expression constructs. Using primers specific to the open reading frame of set2′ in S. pombe (S. pombe GeneDB ID SPAC29B12.02c), full-length set2′ with a C-terminal Flag epitope tag inserted just before the stop codon was PCR amplified from genomic DNA. The resulting product was cloned into either the pCAL-n (Stratagene) bacterial expression vector or the pNS2 yeast expression plasmid. The StepSet2 coding region was sequenced for accuracy. The resulting StepSet2-PNS28 (Set2-Flag) plasmid, which is driven by the ADH1 promoter, was transformed into the S. cerevisiae set2Δ strain BY4742. As a control, the PNS28 plasmid without the open reading frame of set2′ (empty vector) and Set2-Flag were transformed into wild-type and set2Δ BY4742 strains. Wild-type full-length Set2-Flag bacterial and yeast expression constructs have been described previously (48). Transformants were selected on synthetic complete medium (SC) plates lacking uracil.

Expression of recombinant StepSet2. Plasmids expressing either StepSet2-Flag or empty vector were transformed into Escherichia coli BL21(DE3) cells. Five-mL cell cultures were grown to an optical density at 600 nm (OD600) of 0.8 to 1.0 in Luria Broth (LB) medium supplemented with ampicillin (100 μg/ml), followed by addition of 1 mM isopropylthiogalactopyranoside (IPTG) for 3 to 4 h at 30°C. Harvested cells were resuspended in 600 μl lysis buffer (5 mM Tris-HCl, pH 8.0, 0.1% Triton X-1000, 350 mM NaCl, 10% glycerol, 1 mM Mg-lysine, 1 mM phenylmethylsulfonyl fluoride, and 2 μg/ml each leupeptin, aprotinin, and pepstatin). Lysates were prepared by sonication as previously described (48).

In vitro histone methyltransferase assays. Histone methyltransferase assays were performed as previously described with minor modifications (48). Briefly, 1 μl of bacterial lysate was incubated with either 1.25 μg recombinant H3, 5 μg chicken core histones, 5 μg chicken oligomucosomes, or 5 μg H3 synthetic peptide along with 1 μCi S-adenosyl-L-[methyl-3H]methionine ([1H]SAM, 68.8 Ci/mmol, Amersham Biosciences) in methyltransferase buffer (final concentration 50 mM Tris, pH 9.0, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, and 2 μg/ml each leupeptin, aprotinin, and pepstatin) for 30 min at 30°C in a total volume of 10 μl; 2 μl of the reaction was spotted on a Whatman paper, while the remainder was analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) followed by Coomassie staining and fluorography. Identical reactions were performed in parallel using nonradioabeled SAM (40 μM, Sigma) and analyzed by SDS-PAGE followed by Western blotting with the anti-Me3K36 antibody.

Nuclear and whole-cell lystate extracts. For nucleic extractions, wild-type and set2Δ S. pombe strains were grown in 1 liter yeast extract supplemented with adenine (YE) to a final OD600 between 2.0 and 2.5 prior to harvesting. Transformed S. cerevisiae strains were grown to a final OD600 between 2.0 and 2.5 in 200 mL of ochre media lacking uracil and were harvested by vacuum filtration. Nuclei were extracted by lysozyme homogenization from these cell pellets as previously described (12). Yeast whole-cell extracts were prepared from 20 mL cultures grown to a final OD600 between 2.5 and 3.0 as described (8) and only differed in the breaking buffer used for cell disruption (50 mM Tris, pH 8.0, 300 mM NaCl, 1 mM Mg-acetate, 1 mM Imidazole, 0.1% NP-40, 0.5 mM EDTA, 10% glycerol, 2 mM PMSF, phosphatase inhibitor cocktail I [5 μl Sigma], and 2 μg/ml each pepstatin, aprotinin, and leupeptin).

Electrophoresis and immunoblot analyses. SDS-PAGE and Western blot analyses were performed using procedures and reagents from Amersham Biosciences. The anti-H3 mono-K36Me (anti-Me3K36), tri-K36Me (anti-Me3K36), and C terminus of H3 (anti-H3 C-term) rabbit polyclonal antibodies were obtained from Abcam and used at dilutions of 1:1,000, 1:10,000, and 1:20,000, respectively. All other histone modification-detecting antibodies (rabbit) were obtained from Upstate Biotechnology Inc. and used at the following dilutions: 1:3,500 to 1:5,000 for di-K36Me (anti-Me3K36), 1:2,000 for di-K4Me (anti-Me4K4), 1:10,000 for K9Ac (anti Ac K9), and 1:40,000 for tri-K4Me (anti-Me4K4). Mouse monoclonal anti-Flag antibody (M2, Sigma) was used at 1 μg/ml. Anti-polymerase III CT antibodies SWG61 (Unmod CTD) and H3 (Set2) phosphorylation) were used at dilutions of 1:500 and 1:10,000 to 1:30,000, respectively. The immunoglobulin M H14 antibody was detected using hors eradish peroxidase-conjugated donkey anti-mouse immunoglobulin M at 1:5,000 (Jackson ImmunoResearch Laboratories). Typically, 20 to 50 μg of whole-cell extracts or 20 to 100 μg nuclei were resolved on SDS-PAGE gels (8% for RNA Pol II and Flag blots or 13 or 15% for histone modification blots), followed by transfer to polyvinylidine difluoride membranes and immunoblot analyses.

Flag immunoprecipitations. For Flag immunoprecipitations, 2.0 mg of each whole-cell extract was incubated with 12.5 μl of prequillabeled anti-Flag affinity beads (anti-Flag M2 agarose, Sigma) for 2 h at 4°C. After three washes in extraction buffer, the bead-bound proteins were analyzed by immunoblot analysis using the antibodies and dilutions indicated above.

ChIP assays. Chromatin immunoprecipitation (ChIP) assays were performed as previously described (52). Briefly, whole-cell extracts were prepared from formaldehyde-fixed wild-type and set2Δ S. pombe strains grown in 100 μl YE medium to a final OD600 between 1.0 and 1.5. Extracts were sonicated to shear chromatin followed by immunoprecipitation using protein A-Sepharose (Amersham Biosciences) with anti-H3 di-K36Me (anti-Me3K36) at 3 μl/immunoprecipitation. Following washes and DNA elution, cross-links were reversed and DNA was extracted for amplification using standard PCR methods.

Specific regions in the promoter and coding regions of the following genes were amplified: ADE6, PMA1, and ACT1. For a control, we used a primer pair to the K region found in the mating type loci. Primer sequences are available upon request. The results represent the ratio of immunoprecipitated DNA to input DNA normalized to the immunoprecipitation/input ratio from the mating type locus-associated region (K region).

RESULTS

Histone H3 K36 methylation is highly conserved. While K36 methylation has been demonstrated in budding yeast, its presence and relative abundance in other organisms has not been well established. To determine the conservation and relative abundance of K36 methylation in several diverse organisms, we isolated histones from budding yeast, Tetrahymena thermophila, chicken erythrocyte nuclei, and human 293T cells and probed them for K36 methylation using an antidimethyllysine 36 antisemur (anti-H3 Me3K36). For comparison and as a control, we used an antibody specific to dimethylation at histone H3 lysine 4 (anti-H3 Me4K4).

As shown in Fig. 1, we found that K36 dimethylation was present in all of the organisms analyzed, although the relative abundance varied between species. It is interesting that in Tetrahymena thermophila, K36 dimethylation appears to be less abundant compared to the levels of this modification found in yeast, chicken, and humans (Fig. 1): However, a possible reason for this observation may be an inability of the K36 dimethyl antibody to efficiently recognize Tetrahymena thermophila H3. In yeast, chicken, and humans, K36 is immediately preceded by
the amino acid valine, while the predominant form of H3 in *Tetrahymena thermophila* (H3.1) contains an isoleucine that precedes K36 (GG/I_K36KPH versus GG/K_K36KPH). Thus, this amino acid substitution may decrease this antibody’s ability to effectively recognize K36 methylation in the context of its surrounding residues in *Tetrahymena thermophila*. Nonetheless, mass spectrometry analysis confirms that K36 is indeed mono- and dimethylated in this organism (C. D. Allis and D. Hunt, personal communication), although the relative amounts of these methyl forms in *Tetrahymena thermophila* H3 are not known.

In addition to these results, previous studies have shown the existence of K36 methylation in humans, chicken, and sea urchin by protein sequencing (17). Furthermore, we and others have determined that K36 is also methylated in *Drosophila melanogaster*, *Neurospora crassa*, and *Caenorhabditis elegans* (B.D.S., unpublished results) (16). Thus, K36 methylation is found in a broad range of distinct eukaryotes.

With the finding that K36 methylation is highly conserved, we next asked whether Set2 homologs could be identified in these different organisms. Using the AWS (associated with SET), SET, and post-SET domains (amino acids 63 to 260) of the *S. cerevisiae* Set2 (ScSet2) protein as bait in a PSI-BLAST search, we found a significant number of proteins bearing similar sequence structures to Set2, and assembled them in a hierarchical family tree (Fig. 2). As documented in the figure, the ScSet2 protein was most similar to the *S. pombe* and *Neurospora crassa* (NCU00269.1) Set2 proteins (33% and 43% sequence identity, respectively) (34). While not as highly conserved, the domain structure of ScSet2 is found in a number of other proteins found in a variety of diverse organisms.

Strikingly, in addition to the AWS, SET, post-SET, and WW domains, more complex eukaryotes have a large number of additional domains and sequences such as PHD fingers and HMG domains, indicating that these putative Set2 homologs may carry out additional chromatin-related functions. It is notable that the mouse homolog of human NSD1 (Nsd1) has been shown to mediate K36 methylation in vitro (37), suggesting that the other proteins listed in Fig. 2 may be bona fide K36-methylating homologs. However, it was not known whether any of these proteins methylate K36 and associate with Pol II.

*S. pombe* Set2 is a robust methyltransferase specific for K36. To determine if the link between K36 methylation and transcription elongation might be conserved, we characterized the Set2 protein thought to be responsible for K36 methylation in *S. pombe*. We chose to focus on *S. pombe* because many proteins in this organism have been found to be more similar to their mammalian counterparts than to their complements in *S. cerevisiae* (44). In addition, the role of K36 methylation in this organism has not been investigated.

We first asked whether this protein is an active histone methyltransferase (HMT) and whether it catalyzes K36 methylation. To determine this, we cloned the *S. pombe* protein into a pCAL-n expression construct, expressed it in *E. coli*, and tested the recombinant protein in HMT assays using [3H]SAM as a cofactor. As shown in Fig. 3A, SpSet2 showed a robust HMT activity towards nucleosomal substrates and, to a lesser extent, free core histones in filter binding assays. In contrast, this enzyme showed little activity towards free histone H3 (Fig. 3A).

To determine the histone specificity of this methyltransferase, a portion of the HMT assays involving nucleosomal substrates were electrophoresed on a 15% SDS-PAGE gel and examined by fluorography. The results revealed that histone H3 was the only histone methylated (Fig. 3B). We next performed “cold” HMT assays with SpSet2 using unlabeled cofactor, followed by Western blot analysis with an antibody specific for K36 dimethylation to determine if SpSet2 was specific for K36. The results showed a significant immunoreactivity towards K36 dimethylation in the presence of SpSet2 (Fig. 3C). In contrast, no immunoreactivity was witnessed after these HMT assays with antibodies directed against either H3 lysine 79 dimethylation or K4 dimethylation (data not shown).

To further verify the site specificity of SpSet2, we examined H3 synthetic peptides that were either unmodified or trimethylated at K36 in filter binding assays. Although the overall level of activity towards H3 peptides was low (compare [3H] incorporation levels between panels A and D), it was still sufficient to determine whether this activity could be blocked with a K36-methylated peptide. As shown in Fig. 3D, SpSet2 was able to methylate an H3 peptide of residues 27 to 45, but not that of an H3 N-terminal peptide (residues 1 to 20). Importantly, a matched residues 27 to 45 peptide that was trimethylated at K36 was not a substrate (Fig. 3D). These data demonstrate that SpSet2 is a robust nucleosome-selective HMT specific for K36 methylation.

**Set2 and K36 methylation are associated with transcription elongation in *S. pombe***. We next asked whether SpSet2 is responsible for in vivo K36 methylation in *S. pombe* and whether it associates with elongating Pol II. To address the first point, we deleted the *set2*+ gene from *S. pombe* and used these cells, along with the wild-type control, to generate purified nuclei for subsequent Western blot analyses. As shown in Fig. 4A, deletion of *set2*+ resulted in a complete abolishment of K36 methylation (mono-, di-, and trimethylation), but not K4 methylation or H3 K9 acetylation, in bulk histones, indicating that...
FIG. 2. Conservation of Set2 proteins among eukaryotes. (A) Phylogenetic tree of Set2 and its putative homologs. Set2 proteins were identified in different species by using PSI-BLAST and then clustered into groups based on amino acid sequences using the phylogenetic analysis program Clustal X. ExPASy/SIB accession numbers:

- S. cerevisiae Sc_SET2, P46995;
- S. pombe Sp_SET2, O14026;
- N. crassa Nc-NCU00269.1, Q7RZU4;
- Homo sapiens Hs_HIF-1, Q9BYW2;
- Homo sapiens Hs_HSPC069, Q9NZW9;
- Drosophila melanogaster Dm_CG1716, Q9VYD1;
- Homo sapiens Hs_NSD1, Q96L73;
- Homo sapiens Hs_WHSC1L1, Q9BZ95;
- Homo sapiens Hs_WHSC1, O96028;
- C. elegans Ce_C43E11.3, Q8I7H3;
- C. elegans Ce_K09F5.5, Q21404.

The scale bar equals a distance of 0.05 amino acids. The asterisk indicates that this protein is also known as HYPB.

(B) Schematic domain representation of Set2 proteins identified from the alignment in A. Protein names and lengths in amino acids are noted beneath each protein.
SpSet2 is the sole enzyme in fission yeast responsible for this modification. We also examined the set2 deletion (set2Δ) strain for growth defects and found that while set2Δ cells grew normally on rich YEA medium, they showed a strong growth defect in synthetic medium (EMM), which is nutrient depleted compared to YEA (Fig. 4B). These data reveal an important role for Set2 in cell growth under deprived nutrient or stressed conditions. Similar slow-growth phenotypes on minimal medium have been described for the deletion of other factors involved in transcription and translation (2, 45).

To determine if the SpSet2 enzyme would be associated with Pol II, we tagged SpSet2 at its C terminus with a triple Flag epitope (SpSet2-3Flag) and then used this epitope to perform coimmunoprecipitation experiments to monitor the association of unmodified or hyperphosphorylated Pol II. The different forms of Pol II were monitored using antibodies 8WG16 and H14, which recognize unmodified and Ser5 phosphorylated CTD, respectively. As shown in Fig. 4C, immunoprecipitation of SpSet2-3Flag resulted in strong immunoreactivity of the Ser5-phosphorylated CTD form of Pol II. No unmodified Pol II could be detected in these immunoprecipitates, although unmodified Pol II could be readily detected in the input extracts. These data demonstrate that SpSet2 is associated with the elongating form of Pol II in S. pombe. This result is also consistent with the finding that a region in the C terminus of the SpSet2 protein contains similarity (17/42% identity/similarity) to a region in ScSet2 that was found to mediate association of Set2 with the phosphorylated polymerase (22).

Next, we asked whether K36 methylation is associated with the transcribed regions of active genes in S. pombe. To address this, we used a K36 dimethylation-specific antiserum in ChIP assays to examine the abundance and distribution of this modification over genes. Consistent with observations in budding yeast (25, 39, 52), we found that K36 methylation was highly enriched over the transcribed regions of several active genes tested (Fig. 5). In contrast, nontranscribed regions of telomeric and mating type loci were found to be devoid of this methyl mark (data not shown and Fig. 5). These data strongly suggest that K36 methylation, mediated by SpSet2, is associated with the elongation process in S. pombe.

Given the strong similarities found between the budding and fission yeast Set2 proteins, we finally asked if the fission yeast K36-methylating enzyme could complement the loss of Set2 in budding yeast cells. To examine this, we cloned the S. pombe set2 gene, containing a C-terminal Flag tag, into a budding yeast expression construct (under the control of the ADH1 promoter) and expressed this protein in set2Δ cells. As a control, a similar expression construct containing the budding
yeast SET2 gene was included. As shown in Fig. 6, full-length SpSet2 could be readily detected by Western blot analysis using an anti-Flag antibody. The S. pombe protein runs with a slower migration compared to the budding yeast Set2, as this protein is slightly larger than its budding yeast counterpart.

We then purified nuclei from these strains and examined the levels of K36 methylation on bulk histones. Significantly, we found that the S. pombe Set2 protein could restore K36 methylation in set2Δ cells, as this protein is slightly larger than its budding yeast counterpart. This result suggests that SpSet2 forms a stable interaction with Pol II in budding yeast. To examine this idea further, we performed similar coimmunoprecipitation studies as described above and found that SpSet2 efficiently associates with the elongating form of Pol II, similar to its budding yeast counterpart. This result shows that these enzymes are interchangeable, thereby supporting the notion that Set2 and K36 methylation is functionally conserved.

**DISCUSSION**

Like the conservation found between histone protein sequences among eukaryotes, their covalent modifications are also highly conserved (17). Yet, a looming question has been whether these modifications perform the same functions in all of these different organisms or have distinct functions that have arisen through evolutionary change. To date, several sites of histone methylation have been associated with active transcription. These include the methylation of H3 at lysines 4, 36, and 79 (13, 15, 25, 29, 33, 42, 49). In S. cerevisiae, the enzymes responsible for K4 and K36 methylation have been found associated with the elongating form of Pol II (15, 42). This intriguing observation implies a novel role for histone methylation in the elongation phase of transcription. However, whether these enzymes are conserved and have similar functions in organisms outside of budding yeast has not been fully investigated.

In this report, we characterize the fission yeast enzyme responsible for K36 methylation and provide evidence that this modification in S. pombe is coupled with the transcription elongation process. Given budding yeast is evolutionarily distinct from fission yeast, this result suggests that Set2 has a conserved function in transcriptional regulation. While our study has focused on K36 methylation, several studies have characterized Set1 homologs responsible for K4 methylation outside of budding yeast (8, 9, 30, 32, 51). Although the link between K4 methylation and transcription elongation in these
FIG. 5. Set2-mediated K36 methylation is preferentially associated with the transcribed regions of active S. pombe genes. Left panels A to C: Chromatin immunoprecipitation assays were used to monitor the location of K36 dimethylation on actively transcribed genes (ADE6, ACT1, and PMA1) in wild-type and set2Δ strains using an H3 Me2K36-specific antibody. DNA from enriched precipitates (IP) were isolated and used in PCRs with promoter- and coding region-specific primer pairs for the indicated genes. A DNA fragment from the silent mating type loci (K region) of S. pombe known to lack modifications associated with active genes (H3 K4 methylation and H3 K14 acetylation) (34) was used as a control to normalize and calculate the relative enrichment of gene sequences in immunoprecipitated samples. Right panels: Quantification of the ChIP results shown in A to C. Relative enrichment values shown on the y axes were calculated by dividing the ratio of band intensities for immunoprecipitated DNA K region with the ratio of intensities for the input DNA K region. Gels and graphs are representative experiments from three independent repeats.
organisms is not well defined, there are a number of similarities found between Set1 and K4 methylation among eukaryotes that suggest a conserved role for this modification similar to what is found for Set2/K36 methylation. First, Set1-mediated K4 methylation is associated primarily with euchromatic regions found between Set1 and K4 methylation among eukaryotes. Second, comparative studies of K4 methylation suggest a conserved role for this modification similar to what is found for Set2/K36 methylation. Given that not all Set2 homologs contain this domain, we speculate that K36 methylation will have a broad range of activities in chromatin in addition to a conserved role with the transcribing polymerase.

In summary, we demonstrate that SpSet2 is a true ortholog of the budding yeast Set2 enzyme and that this enzyme and K36 methylation are linked to the transcription elongation process in S. pombe. While our characterization studies are limited to S. pombe, an accompanying paper shows similar findings for the Neurospora crassa Set2 homolog (1). Furthermore, a link between K36 methylation and CTD phosphorylation has been suggested in Caenorhabditis elegans (16), and recent evidence shows a correlation of K36 methylation with active genes in metazoans (6). Taken together, these results indicate a highly conserved role for K36 methylation in transcriptional regulation.

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REFERENCES

1. Adhvaryu, K. K., S. A. Morris, B. D. Strahl, and E. U. Selker. 2005. Methylation of histone H3 lysine 36 is required for normal development in Neurospora crassa. Eukaryot. Cell 4:1455–1464.
2. Akyoshi, Y., J. Clayton, L. Phan, M. Yamamoto, A. G. Hinnebusch, Y. Watanabe, and K. Asano. 2001. Fission yeast homolog of murine Int-6 protein, encoded by my mouse mammary tumor virus integration site, is associated with the conserved core subunits of eukaryotic translation initiation factor 3. J. Biol. Chem. 276:10056–10062.
3. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403–410.
4. Briggs, S. D., M. Bryk, B. D. Strahl, W. L. Cheung, J. K. Davie, S. Y. Dent, F. Winston, and C. D. Allis. 2001. Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and DNA silencing in Saccharomyces cerevisiae. Genes Dev. 15:3286–3295.
5. Byrd, K. N., and A. Shearn. 2003. ASHI, a Drosophila trithorax group protein, is required for methylation of lysine 4 residues on histone H3. Proc. Natl. Acad. Sci. USA 100:11535–11540.
6. Cao, R., and Y. Zhang. 2004. The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Curr. Opin. Genet. Dev. 14:155–164.
7. Cosgrove, M. S., J. D. Boeke, and C. Wolberger. 2004. Regulated nucleosome mobility and the histone code. Nat. Struct. Mol. Biol. 11:1037–1043.
12. Edmondson, D. G., M. M. Smith, and S. Y. Roth. 1996. Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. Genes Dev. 10:1247–1259.
13. Gerber, M., and A. Shilatifard. 2003. Transcriptional elongation by RNA polymerase II and histone methylation. J. Biol. Chem. 278:26303–26306.
14. Grewal, S. L., and J. C. Rice. 2004. Regulation of heterochromatin by histone methylation and small RNAs.Curr. Opin. Cell Biol. 16:230–238.
15. Hampsey, M., and D. Reinberg. 2003. Tails of intrigue: phosphorylation of RNA polymerase II mediates histone methylation. Cell 113:429–432.
16. Han, Z., J. R. Saam, H. P. Adams, S. E. Mango, and J. M. Schumacher. 2003. The C. elegans Toused-like kinase (TLK-1) has an essential role in transcription. Curr. Biol. 13:1921–1929.
17. Holde, K. E. 1988. Chromatin. Springer-Verlag, New York, N.Y.
18. Hughes, C. M., O. Rozenblatt-Rosen, T. A. Milne, T. D. Copeland, S. S. Koh, K. Hayward, J. L. Hess, and M. Meyerzon. 2004. Menin associates with a trithorax family histone methyltransferase complex and with the boxc locus. Mol. Cell 13:587–597.
19. Iizuka, M., and M. M. Smith. 2003. Functional consequences of histone modifications. Curr. Opin. Genet. Dev. 13:154–160.
20. Jeanmougin, F., J. D. Thompson, M. Gouy, D. G. Higgins, and T. J. Gibson. 1998. Multiple sequence alignment with Clustal X. Trends Biochem. Sci. 23:403–405.
21. Jenuwein, T., and C. D. Allis. 2001. Translating the histone code. Science 293:1074–1080.
22. Kizer, K. O., H. P. Phatnani, Y. Shibata, H. Hall, A. L. Greenleaf, and B. D. Strahl. 2005. A novel domain in Set2 mediates RNA polymerase II interaction and couples histone H3 K6 methylation with transcript elongation. Mol. Biol. Cell 25:3305–3316.
23. Kouzarides, T. 2002. Histone methylation in transcriptional control. Curr. Opin. Genet. Dev. 12:198–209.
24. Krogan, N. J., J. Dover, A. Wood, J. Schneider, J. Heidt, M. A. Boateng, K. Dean, O. W. Ryan, A. Golshani, M. Johnston, J. F. Greenblatt, and A. Shilatifard. 2003. The Pol1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. Mol. Cell. 11:721–729.
25. Krogan, N. J., M. Kim, A. Tong, A. Golshani, G. Gagne, V. Canadian, D. P. Richards, R. K. Beattie, A. Emili, C. Boone, A. Shilatifard, S. Buratowski, and J. Greenblatt. 2003. Methylation of histone H3 by Set2 in Saccharomyces cerevisiae is linked to transcriptional elongation by RNA polymerase II. Mol. Cell. Biol. 23:4207–4218.
26. Lee, D. Y., C. Teysier, B. D. Strahl, and M. R. Stellacci. 2004. Role of protein methylation in regulation of transcription. Endocrinol. Rev. 26:637–651.
27. Li, B., L. Howe, S. Anderson, J. R. Yates 3rd, and J. L. Workman. 2003. The Set2 histone methyltransferase functions through the phosphorylated carboxyl-terminal domain of RNA polymerase II. J. Biol. Chem. 278:8897–8903.
28. Li, J., D. Moazed, and S. P. Gygi. 2002. Association of the histone methyltransferase Set2 with RNA polymerase II plays a role in transcription elongation. J. Biol. Chem. 277:19383–19388.
29. Litt, M. D., M. Simpson, M. Gaszner, C. D. Allis, and G. Felsenfeld. 2001. Correlation between histone lysine methylation and developmental changes at the chicken beta-globin locus. Science 293:2453–2455.
30. Milne, T. A., S. D. Briggs, H. W. Brock, M. E. Martin, D. Gibbs, C. D. Allis, and J. L. Hess. 2002. MLL targets SET domain methyltransferase activity to Hox gene promoters. Mol. Cell. 10:1107–1117.
31. Ng, H. H., F. Robert, R. A. Young, and K. Struhl. 2003. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol. Cell 11:79–79.
32. Nishioka, K., S. Chikub, K. Sarma, H. Erdjument-Bromage, C. D. Allis, P. Tempst, and D. Reinberg. 2002. Set1, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. Genes Dev. 16:479–489.
33. Noma, K., C. D. Allis, and S. I. Grewal. 2001. Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. Science 293:1150–1155.
34. Noma, K., and S. I. Grewal. 2002. Histone H3 lysine 4 methylation is mediated by Set1 and promotes maintenance of active chromatin states in fission yeast. Proc. Natl. Acad. Sci. USA 99(Suppl. 4):16438–45.
35. Perriere, G., and M. Gouy. 1995. WWW-query: an on-line retrieval system for biological sequence banks. Bioinformatics 7:364–369.
36. Peterson, C. L., and M. A. Laniel. 2004. Histones and histone modifications. Curr. Biol. 14:R546–R556.
37. Rayasam, G. V., O. Wendling, P. O. Angrand, M. Mark, K. Niederreither, L. Song, T. Lerouve, G. L. Hager, P. Chambon, and R. Losson. 2003. NSD1 is essential for early post-implantation development and has a catalytically active SET domain. EMBO J. 22:3153–3163.
38. Roguev, A., D. Schaft, A. Shevchenko, R. Aasland, A. Shevchenko, and A. F. Stewart. 2003. High conservation of the Set1/Rad6 axis of histone 3 lysine 4 methylation in budding and fission yeasts. J. Biol. Chem. 278:4878–4893.
39. Saito, D., A. Roguev, K. M. Katovic, A. Shevchenko, M. Sarov, A. Shevchenko, K. M. Neugebauer, and A. F. Stewart. 2003. The histone 3 lysine 36 methyltransferase, SET2, is involved in transcriptional elongation. Nucleic Acids Res. 31:2475–2482.
40. Siliprandi, M. 2000. Where does fission yeast sit on the tree of life? Genome Biol. 1:REVIEW0010.
41. Smith, K. N., L. Iwanejko, S. Laefelt, F. Fabre, and A. Nicolas. 1999. Disruption and functional analysis of seven ORPs on chromosome IV: YDL107w, YDL101w, YDL096c, YDL186w (APC11), YDL056c (MED2) and YDL033w (MCD1). Yeast 15:1255–1267.
42. Strahl, B. D., and C. D. Allis. 2000. The language of covalent histone modifications. Nature 403:41–45.
43. Strahl, B. D., S. S. Briggs, C. J. Brame, J. A. Caldwell, S. S. Koh, H. Ma, R. G. Cook, J. Shabanowitz, D. F. Hunt, M. R. Stellacci, and C. D. Allis. 2001. Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. Curr. Biol. 11:996–1006.
44. Siprich, M. 2000. What does fission yeast sit on the tree of life? Genome Biol. 1:REVIEW0010.
45. Smith, K. N., L. Iwanejko, S. Laefelt, F. Fabre, and A. Nicolas. 1999. Disruption and functional analysis of seven ORPs on chromosome IV: YDL107w, YDL101w, YDL096c, YDL186w (APC11), YDL056c (MED2) and YDL033w (MCD1). Yeast 15:1255–1267.
46. Strahl, B. D., and C. D. Allis. 2000. The language of covalent histone modifications. Nature 403:41–45.
47. Strahl, B. D., S. S. Briggs, C. J. Brame, J. A. Caldwell, S. S. Koh, H. Ma, R. G. Cook, J. Shabanowitz, D. F. Hunt, and C. D. Allis. 2001. SET2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. Mol. Cell. Biol. 22:1298–1306.
48. Zhang, Y., and D. Reinberg. 2001. Transcription regulation by histone methyltransferases and histone modifications. Curr. Opin. Genet. Dev. 11:135–140.