

COMPASS, a Histone H3 (Lysine 4) Methyltransferase Required for Telomeric Silencing of Gene Expression*

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The trithorax (Trx) family of proteins is required for maintaining a specific pattern of gene expression in some organisms. Recently we reported the isolation and characterization of COMPASS, a multiprotein complex that includes the Trx-related protein Set1 of the yeast *Saccharomyces cerevisiae*. Here we report that COMPASS catalyzes methylation of the fourth lysine of histone H3 *in vitro*. Set1 and several other components of COMPASS are also required for histone H3 methylation *in vivo* and for transcriptional silencing of a gene located near a chromosome telomere.

Alteration of chromatin by covalent modification of histone proteins is central to the regulation of gene expression in eukaryotic organisms (1–3). Acetylation of histones H3 and H4 are the best characterized covalent modifications of histones and have wide ranging effects on gene expression (1–4). Histone phosphorylation is important for transcriptional activation, condensation of chromosomes during mitosis and meiosis, and regulation of cell division (5, 6).

Recently, Lys⁴ and Lys⁹ of histone H3 and Arg³ of histone H4 were found to be methylated (7, 8). An enzyme that catalyzes

the methylation of Lys⁹ of histone H3 was identified in mammals (human SUV39H1 and mouse Suv39 h1) and found to be the homologue of *Drosophila Su(var)3–9*, involved in silencing of heterochromatic gene expression, and of *Schizosaccharomyces pombe clr4*, which is involved in silencing of expression of the mating-type locus and genes near centromeres (9–12). The catalytic domain of *Su(var)39* is thought to be its SET domain, which takes its name from the *Drosophila* proteins *Su(var)3–9*, *Enhancer of zeste (E(z))*, and *trithorax (trx)* (13, 14).

We recently identified COMPASS, a multiprotein complex that contains the *Saccharomyces cerevisiae* SET domain-containing protein Set1 and another yeast protein related to the human Trx protein ASH2 (15). Here we show that COMPASS catalyzes methylation of lysine 4 of histone H3 and is required for transcriptional silencing of genes located near chromosome telomeres.

EXPERIMENTAL PROCEDURES

Materials—Media and reagents were purchased from Sigma. Western development reagents were purchased from ICN ImmunoBiologicals (Irvine, CA). S-Adenosylmethionine was purchased from Fisher.

Yeast Strains—The role of the *CPS* genes (encoding proteins that comprise COMPASS) in telomere-associated silencing of gene expression was determined by disrupting them in the strain UCC1001 (*MATa ura-52 his3D200 ade2–101 lys2–801 trp1-D1 leu2-D1 TELVIIadh4::URA3*), which carries the *URA3* gene near the left telomere of chromosome 7. Each *CPS* gene was replaced by the *KanMX* gene (16) by transforming yeast to G418 resistance with a PCR product of *cps::KanMX* (amplified from the *S. cerevisiae* yeast gene knockout collection (17) that includes 45 nucleotides of DNA sequence flanking each side of the *cps::KanMX* gene disrupted (to provide for homologous recombination with the target *CPS* gene)). Each *cps::KanMX* mutant was confirmed by a PCR test using, as primers, one oligonucleotide within *KanMX* and one oligonucleotide flanking the disrupted *CPS* gene.

Preparation of Yeast Cell Extracts and Test for Histone H3 Methylation—Yeast cells were grown in YPD overnight to mid-log phase. Cells were washed with distilled water, pelleted, and resuspended in lysis buffer (20 mM Tris at pH 7.5, 50 mM KCl, 1 mM EDTA, 0.1% Nonidet P-40, 1 mM dithiothreitol, and fresh protease and phosphatase inhibitors (1 μg/ml aprotinin, leupeptin, and pepstatin A, 1 mM phenylmethylsulfonyl fluoride, 1 μM microcystin-LR, 2 mM phenylmethylsulfonyl fluoride). Cells were then disrupted by vortexing with glass beads (0.5 mm; Biospec Products) for 15 min at 4 °C. The bottoms of the microcentrifuge tubes were punctured, and cell extracts were recovered into a larger tube by brief centrifugation in a microcentrifuge. The lysate was clarified by centrifugation at 20,000 × g for 30 min, subjected to SDS-PAGE, transferred to nitrocellulose membrane, and probed with anti-methylhistone antisera (United States Biochemical, Inc., catalog number 07-030) at 1:1000 dilution, followed by detection of the bound antibody with horseradish peroxidase conjugated to anti-rabbit IgG secondary antibodies (1:10,000 dilution).

RESULTS

Methylation of Lysine 4 of Histone H3—We tested for the presence of methylated histones H3 and H4 by employing antibodies specific for methylated Lys⁹ of histone H3, Lys⁴ of histone H3, and Arg³ of histone H4. Probing yeast extracts separated by SDS-PAGE with antiserum specific to these modifications reveals that only Lys⁴ of histone H3 is methylated in *S. cerevisiae* (Fig. 1A). The protein indicated is indeed [methyl-Lys⁴]H3, because histone H3 purified from cells via 6 histidine residues placed at its C terminus is recognized by this antiserum and exhibits approximately the same mobility in SDS-PAGE as the protein inferred to be [methyl-Lys⁴]histone H3 (Fig. 1C). We observed methylation of histone H3 Lys⁴ and histone H4 Arg³ in extracts obtained from both mammalian and *Drosoph-*

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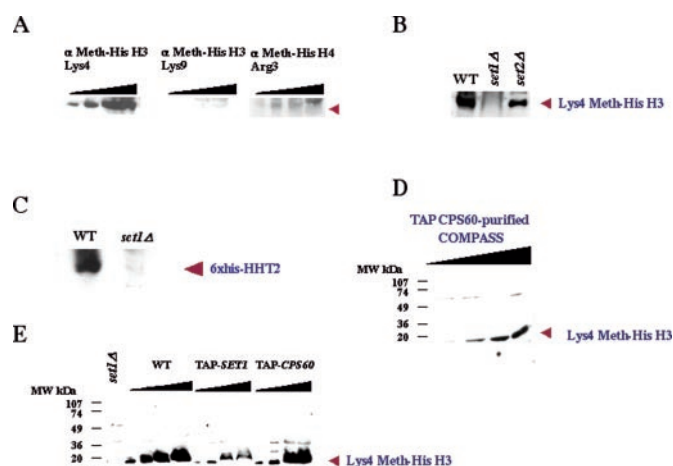


FIG. 1. Methylation of the fourth lysine of histone H3 in *S. cerevisiae*. 32 A, whole cell extracts from *S. cerevisiae* were subjected to a 16% SDS-PAGE, blotted to nitrocellulose membrane, and probed with polyclonal antisera specific for methylated Lys⁹ of histone H3, Lys⁴ of histone H3, and Arg³ of histone H4. B, whole cell extracts from wild-type *S. cerevisiae* or yeast strains missing SET1 or SET2 were tested for the presence of Lys⁴-methylated histone H3. Cell extracts were subjected to 16% SDS-PAGE, blotted to nitrocellulose membrane, and probed with polyclonal antisera specific for Lys⁴ of histone H3. C, to confirm that we have correctly assigned the SDS-PAGE band corresponding to Lys⁴-methylated H3, the histone H3 gene (*HHT2*) was tagged at its C terminus with six histidine residues in both wild-type and *set1Δ* cells. Whole cell extracts of these cells were treated with nickel-agarose beads, and the bound fraction was subjected to a 16% SDS-PAGE, blotted to nitrocellulose membrane, and probed with the anti-[methyl-Lys⁴]H3 polyclonal antiserum. D, increasing amounts of Cps60 TAP-purified COMPASS were tested for *in vitro* histone H3 Lys⁴ methyltransferase activity. Reaction mixtures containing cold *S*-adenosylmethionine, recombinant histone H3, and appropriate buffers (9) without or with increasing concentration of COMPASS were incubated at 37 °C for 1 h. Reactions were stopped by the addition SDS-PAGE sample loading buffer and subjected to a 16% SDS-PAGE. The appearance of Lys⁴-methylated histone H3 was detected by Western analysis employing polyclonal antisera specific to Lys⁴ of histone H3.

ila cells (data not shown), suggesting that these modifications are specific to larger eukaryotic organisms or present in *S. cerevisiae* at very low abundance. Allis and co-workers also observed this recently and suggested that high levels of H3 Lys⁴ methylation may reflect a fundamental difference between single-celled and multicellular organisms in the “ground state” of their chromatin (18).

The Set1 Subunit of COMPASS Is Required for Methylation of the Fourth Lysine of Histone H3 *In Vivo*—Recently the SET domains of the *Su(var)3–9* family of proteins of *Drosophila* and mammalian G9a protein were shown to catalyze the methylation of the Lys⁹ of histone H3 *in vitro* (9, 19). We therefore tested whether the Set1 protein is required for methylation of histone H3 Lys⁴ *in vivo*. Indeed, this modification is absent in cells missing the Set1 subunit of COMPASS (Fig. 1, B and C). Removal of SET2 (Fig. 1B) or the genes encoding other SET domain-containing proteins (data not shown) did eliminate histone H3 methylation.

Set1 Catalyzes Methylation of Lys⁴ of Histone H3 *In Vitro*—Although we did not detect *in vitro* histone methyltransferase activity of COMPASS purified via the TAP¹ epitope (15) placed on Set1, COMPASS purified using epitope-tagged Cps60 possesses H3 Lys⁴ HMT activity *in vitro* (Fig. 1D). To determine whether tagging SET1 on its C-terminal domain alters its catalytic activity, we tested yeast cell extracts from wild-type, SET1-TAP, and CPS60-TAP. Set1 tagged on its C-terminal

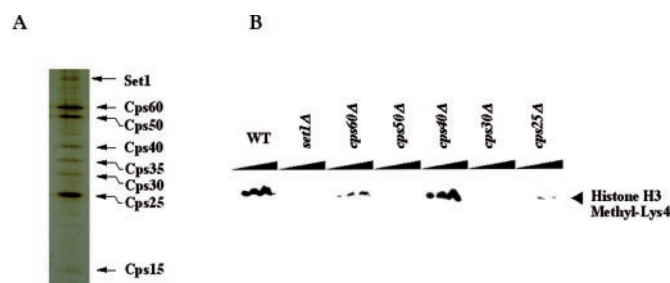


FIG. 2. Analysis of the subunits of COMPASS essential for histone H3 Lys⁴ methylation *in vivo*. A, the purified core COMPASS consists of Set1, Cps60, Cps50, Cps40, Cps35, Cps30, Cps25, and Cps15. TAP-tagged Cps60 was purified as described previously (15) and applied to 16% SDS-PAGE, and COMPASS subunits were visualized by silver staining. Cps15 is not detected in COMPASS separated on a lower percentage SDS-PAGE gel. B, the presence of methylation of Lys⁴ of histone H3 in yeast strains missing genes encoding subunits of COMPASS was determined by subjecting an increasing concentration of whole cell extracts from wild-type and nonessential *cps* mutants to 16% SDS-PAGE followed by immunoblotting as described above. We observed the same pattern of H3 Lys⁴ methylation in the *cps* mutants from the yeast gene knockout strain collection (data not shown).

domain is crippled and loses about 50–70% of its activity in methylating Lys⁴ of histone H3 *in vivo* (Fig. 1E).

Some Subunits of COMPASS Are Essential for Histone H3 Lys⁴ Methylation—The eight subunits of purified COMPASS analyzed by SDS-PAGE are shown in Fig. 2A. We tested six nonessential subunits of COMPASS for a role in histone H3 Lys⁴ methylation by assaying for this modification in yeast strains missing the various *CPS* genes. SET1, CPS50, and CPS30 appear to be required for H3 Lys⁴ methylation; *cps60* and *cps25* mutants have detectable, but apparently reduced, methylation activity (Fig. 2). Cps40 is not required for this histone modification.

Correlation between Methylation of Histone H3 Lys⁴ and Gene Silencing at the Telomeres—It has been shown that Set1 is required for full silencing of expression of a gene located near chromosome telomeres or in the rDNA repeat (18, 20). In our previous study we tested *cps* mutants for telomere-associated gene silencing by observing the ability of a *URA3* gene located near the left telomere of chromosome 7 to confer a Ura⁺ phenotype. However, we have come to believe that this test is not an entirely accurate assay for telomeric silencing. Therefore, we sought to confirm our previous results by testing the ability of the telomere-associated *URA3* gene to confer sensitivity to 5-fluoro-orotic acid (5-FOA), as described previously (20). In agreement with our previous results, SET1, CPS50, CPS30, and CPS25 all appear to be required for normal silencing of this gene. In contrast to our previous results, *cps40* and *cps60* (*bre2*) mutants appear to retain some ability to silence the telomere-associated *URA3* gene. Telomeric gene silencing correlated with the methylation activity of COMPASS; the three *cps* mutants with some methylation activity (*cps25*, *cps40*, and *cps60*) appear to retain at least some gene silencing ability and *cps* mutants lacking H3 Lys⁴ methylation appear not to silence the expression of *URA3* located near a telomere.

DISCUSSION

The Set1 protein of yeast is similar to the *Drosophila* and human trithorax proteins (Trithorax (Trx) and MLL, respectively) (21–24). Our understanding of the role of this class of proteins in regulation of gene expression and development is rudimentary. Trx is a putative DNA-binding protein that seems to be a positive regulator of gene expression (24). Mutations affecting MLL result in the development of hematological malignancies (24). Our characterization of the Set1-containing protein complex we call COMPASS is a first step toward un-

¹ The abbreviations used are: TAP, tandem affinity purification; HMT, histone methyltransferase; 5-FOA, 5-fluoro-orotic acid.

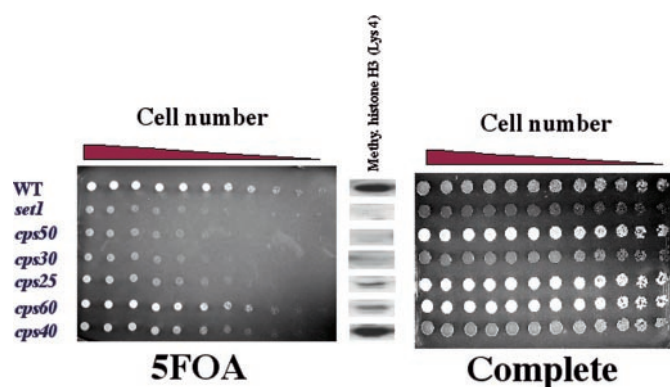


FIG. 3. Deletion of subunits of COMPASS results in a defect in silencing of gene expression at telomeres in a 5-FOA-dependent manner. Genes encoding six nonessential subunits of COMPASS were deleted in a strain (UCC1001, Ref. 19) harboring *URA3* near the left telomere of chromosome 7 as a reporter of telomeric gene silencing. The resulting *cps* mutants were tested for growth in the presence of 5-FOA (20). Wild-type cells silence expression of the telomere-associated *URA3* gene and are therefore resistant to 5-FOA. Cells defective for telomeric gene silencing have increased expression of *URA3* and hence are sensitive to 5-FOA (20). Two-fold serial dilutions of cultures (from approximately 5×10^4 to 50 cells) were spotted on minimal glucose plates containing (left panel) or lacking (right panel) 5-FOA. These plates were incubated at 30 °C for 40 (± 4) h.

Understanding the function of SET domain-containing proteins. We and others (15, 18, 25) have now provided evidence that COMPASS is a histone methyltransferase that catalyzes methylation of Lys⁴ of histone H3.

Set1 is the COMPASS subunit likely responsible for its catalytic activity. It appears that tagging Set1 on its C-terminal domain cripples its methyltransferase activity both *in vivo* and *in vitro* (Fig. 1E). Although it was recently demonstrated that the Set domain of mammalian Trx (MLL) lacks histone methyltransferase activity *in vitro* (9), it is possible the recombinant protein used in that study is defective due to the lack of interacting proteins. Some of the other COMPASS subunits that are required for its methyltransferase activity *in vivo* may play a role in either substrate recognition or proper folding of the Set domain.

It has been reported that the Trx-related Ash1 protein is a methyltransferase specific for H3 lysines 9 and 27 (25). The observation that Cps60, which is similar to Ash2, is important for the methyltransferase activity of COMPASS suggests a common role for the Trx group of proteins as histone methyltransferases. Since the methylation activity of COMPASS seems to be roughly correlated with silencing of telomeric gene expression (Fig. 3), methylation of the Lys⁴ of histone H3 is implicated in the establishment and/or maintenance of telomeric gene silencing.

Recently, a few key pieces of evidence suggest that modification of histone H3 at its fourth lysine residue facilitates transcriptional activation (26). First, methylation of Lys⁴ of histone H3 is preferentially associated with regions of chromosomes that are transcriptionally active or seem poised for tran-

scription (27, 28). Second, Set1 protein has been linked to expression of several genes in *S. cerevisiae* involved in transcriptional regulation, meiosis, DNA repair, and cell cycle (15, 20, 29). In contrast, COMPASS seems required for silencing of gene expression at telomeres. In addition to our results and those of Pillus and co-workers supporting this idea (15, 20), Allis and colleagues have recently demonstrated that [methyl-Lys⁴]histone H3 is present at the rDNA locus and that this modification is required for silencing of RNA polymerase II transcription of a gene situated within the rDNA (18).

The apparent dual nature of methylation of histone H3 in repression and activation may be explained, at least in part, by the different roles played by this modification in yeast and multicellular eukaryotes. The different roles of this histone modification emphasize the importance of the "histone code" (26) in chromatin structure and regulation of gene expression.

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