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Yeast Jhd2p is a histone H3 Lys4 trimethyl demethylase

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Histone methylation is important in regulating chromatin structure and function. In budding yeast, methylation of histone H3 at Lys4 (H3-K4) is associated with active transcription and is enriched at the 5′ regions of transcribed genes. Here we identify a novel budding yeast JmjC domain–containing H3-K4 demethylase, Jhd2p, that antagonizes the trimethyl modification state and contributes to regulation of telomeric silencing.

Histone methylation on lysine residues contributes to transcriptional regulation, maintenance of genome integrity and epigenetic inheritance[1]. Characterization of individual histone lysine methylation marks has revealed that specific modifications can have very defined functional effects on surrounding chromatin. In addition, each modified lysine can exist in the mono- (me1), di- (me2), or trimethyl (me3) state, increasing the potential complexity of the histone lysine modification system. The effects of histone methylation are mediated largely through recruitment of effector proteins that can recognize regions of differentially modified chromatin[2–5]. In budding yeast (*Saccharomyces cerevisiae*), histone lysine methylation occurs exclusively on histone H3 at Lys4, Lys36 and Lys79 (ref. 6). H3-K4 is methylated by the Set1p methyltransferase during transcriptional initiation, through association with the Ser5-phosphorylated CTD of RNA polymerase II[7]. Although the dynamics of H3-K4 methylation in budding yeast are poorly characterized, some rapid changes in H3-K4 methylation have been reported, suggesting that an active mechanism exists to counteract this modification[8,9].

Recently, JmjC domain–containing enzymes have been shown to directly remove histone lysine methylation via a hydroxylation reaction that requires iron and α-ketoglutarate as cofactors[10,11]. In budding yeast, there are five JmjC domain–containing proteins: Jhd1p, Rph1p, Gis1p, Yjr119Cp and Ecm5p (**Fig. 1a**). Jhd1p has previously been shown to catalyze demethylation of H3-K36me2 and H3-K36me1 (ref. 11), but the potential enzymatic activity of other JmjC domain–containing proteins remains uncharacterized. Bioinformatic analysis has indicated that substitution mutations in important catalytic residues of JmjC domains of Gis1p and Ecm5p render the JmjC domain enzymatically inactive[10]. We have recently demonstrated that Rph1p catalyzes demethylation of H3-K36me3 and H3-K36me2 (R.J.K., K.E.G., G.L. and Y.Z., unpublished data). To determine whether the remaining JmjC domain–containing protein, Yjr119Cp, is a histone demethylase, we expressed recombinant Yjr119Cp in

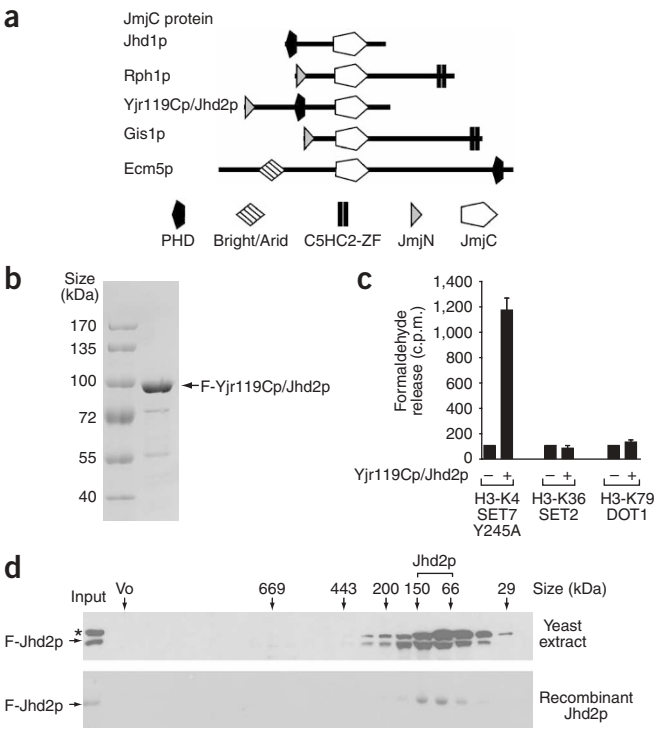

Figure 1 Budding yeast Yjr119Cp (renamed Jhd2p) is an H3-K4 demethylase. (**a**) Schematic illustration of domain architecture of the five JmjC domain–containing proteins in budding yeast. (**b**) Coomassie-stained gel showing affinity-purified recombinant Yjr119Cp/Jhd2p produced in insect cells. (**c**) Histone substrates were labeled using methyltransferase enzymes that modify known histone methylation sites in budding yeast and incubated with recombinant Yjr119Cp/Jhd2p. Histone demethylase activity was monitored as the release of labeled formaldehyde, plotted as a bar graph (error bars show s.d.). Yjr119Cp/Jhd2p specifically demethylates H3-K4me3 and H3-K4me2. (**d**) Top, Flag-Jhd2 (F-Jhd2p) in yeast extract was fractionated by size-exclusion chromatography and identified by Flag-specific western blotting (top gel). Asterisk indicates a cross-reacting band found in yeast extracts. Size-exclusion chromatography molecular weight markers are indicated above the panel. Jhd2p eluted with an apparent molecular weight of 80–100 kDa. Bottom, recombinant Jhd2p was fractionated by size-exclusion chromatography and identified by Coomassie staining. Recombinant Jhd2 eluted with the same apparent molecular weight as endogenous Jhd2p, suggesting Jhd2p is a monomeric protein that does not form a stable high–molecular weight protein complex.

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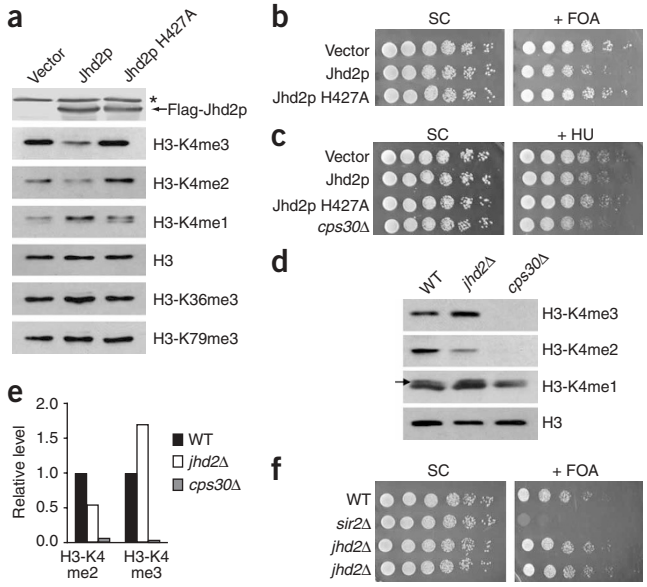

Figure 2 Jhd2 antagonizes H3-K4me3 methylation and regulates telomeric silencing. (**a**) Whole-cell extracts were prepared from strains containing either empty vector or constructs expressing Flag-tagged wild-type Jhd2p or the H427A mutant. Histone methylation levels were analyzed by western blotting using modification-specific antibodies. (**b**) WT Jhd2p or Jhd2p H427A was overexpressed in a strain carrying a silenced telomeric *URA3* reporter gene. After normalization for cell number, each strain was serially diluted in five-fold increments and spotted on SC medium or SC containing 100 μg μl^{-1} FOA. Strains overexpressing Jhd2p show reactivation of the *URA3* gene, evident from growth sensitivity on plates containing FOA. (**c**) WT Jhd2p or Jhd2p H427A was overexpressed in wild-type yeast. Each strain was serially diluted as in **c** and spotted on SC medium or SC containing 100 mM hydroxyurea. Strains overexpressing Jhd2p are sensitive to hydroxyurea, as is evident from reduced growth. A *cps30*Δ strain, which has compromised Set1 enzymatic function, was used as a control for sensitivity to hydroxyurea. (**d**) Whole-cell extracts were prepared from wild-type, *jhd2*Δ and *cps30*Δ strains. H3-K4 methylation levels were analyzed by western blotting using modification-specific antibodies. The *jhd2*Δ strain has increased H3-K4me3 and reduced H3-K4me2. (**e**) Plot of quantified relative changes in **d**. (**f**) Cells from WT, *sir2*Δ and *jhd2*Δ strains were normalized for cell density and spotted in five-fold dilutions onto SC medium or SC medium containing 100 μg μl^{-1} FOA. *sir2*Δ strain is a control for loss of telomeric silencing. Two independent *jhd2*Δ strains show enhanced telomeric silencing, evident from reduced sensitivity to FOA in the *URA3* reporter strain.

insect cells using a baculovirus expression system and purified the recombinant protein by affinity chromatography (**Fig. 1b** and **Supplementary Methods** online). Recombinant Yjr119Cp was incubated with radioactively labeled histone substrates corresponding to all three histone lysine methylation sites in yeast, and demethylase activity was analyzed by release of the labeled reaction product formaldehyde (**Fig. 1c**). Yjr119Cp catalyzed demethylation of substrates labeled by Set7p Y245A, an enzyme capable of producing H3-K4me3 and H3-K4me2 modification states[12], but not substrates labeled in the H3-K36 or H3-K79 positions (**Fig. 1c**). Notably, Yjr119Cp was catalytically inactive toward substrates produced by wild-type Set7p, which generates the H3-K4me1 modification state (data not shown), suggesting that Yjr119Cp targets H3-K4me2/3 but not H3-K4me1. To reflect the protein's newly identified enzymatic activity, we have renamed the *YJR119C* gene as JmjC domain–containing histone demethylase-2 (*JHD2*).

Many chromatin-modifying proteins in yeast are found in large multiprotein complexes with auxiliary proteins that function to target, and in some cases regulate, enzymatic activity[6,13]. To gain insight into potential functional partners of Jhd2p, we sought to analyze whether Jhd2p forms a high–molecular weight multiprotein complex. To this end, extract from a strain containing Flag-tagged Jhd2p was separated by size-exclusion chromatography and Jhd2p-containing fractions were identified by western blot analysis. The molecular weight of Jhd2p, as determined by size exclusion chromatography, is between 80 and 100 kDa, which corresponds to the predicted molecular weight of monomeric Jhd2p (85 kDa; **Fig. 1d**, top). The elution profile of Jhd2p from yeast extracts mirrors the profile of recombinant protein fractionated on the same column, verifying the monomeric nature of this enzyme (**Fig. 1d**, bottom). Therefore, Jhd2p seems to function as an H3-K4 demethylase in the absence of stably associated protein factors.

To examine whether Jhd2 can target demethylation of H3-K4 *in vivo*, wild-type (WT) Jhd2p or Jhd2p with a mutation in a proposed iron-binding site (H427A) were overexpressed in budding yeast, and the resulting H3-K4 methylation states were analyzed with modification-specific antibodies (**Fig. 2a**). Overexpression of WT Jhd2 resulted in a reduction of H3-K4me3 and H3-K4me2 and an increase in H3-K4me1 (**Fig. 2a**, middle lane). Jhd2 demethylase activity was specific for H3-K4, as the levels of H3-K36me3 and H3-K79me3 remained unchanged (**Fig. 2a**). The effect of Jhd2p on H3-K4 methylation was completely dependent on an intact JmjC domain, as mutation of a predicted iron-binding residue abrogated demethylase activity (**Fig. 2a**, right lane). Alteration of H3-K4 methylation levels by deletion of Set1p or the Cps30p component of the Set1 complex causes defects in telomeric silencing and sensitivity to agents that inhibit DNA replication[14–18]. To examine whether elevated levels of Jhd2p result in similar cellular defects, Jhd2p was overexpressed in a strain containing the *URA3* telomeric reporter[19] or treated with the DNA replication–inhibiting agent hydroxyurea (**Fig. 2b**,**c**). Notably, elevated levels of Jhd2 and demethylation of H3-K4 caused partial reactivation of the telomeric *URA3* reporter gene, as evidenced by reduced growth on 5-fluoroorotic acid (FOA)-containing media, and also reduced growth after treatment with hydroxyurea (**Fig. 2b**,**c**). These effects were completely dependent on the enzymatic activity of Jhd2p, they did not occur upon overexpression of catalytically inactive Jhd2p H427A (**Fig. 2b**,**c**). These data indicate that Jhd2p can demethylate H3-K4 *in vivo* to counteract cellular functions mediated by Set1p methylation. Furthermore, overexpression of Jhd2p resulted in DNA replication defects and loss of telomeric silencing similar to strains with perturbed Set1p function[14–18], indicating that these effects are dependent on H3-K4 methylation and not other regulatory properties of the Set1 complex.

To determine whether endogenous Jhd2p contributes to regulation of normal H3-K4 methylation levels, we deleted the *JHD2* gene and analyzed H3-K4 methylation levels using modification-specific antibodies (**Fig. 2d**). In strains lacking Jhd2p, there was an increase in H3-K4me3 (**Fig. 2d**,**e**), whereas H3-K4me2 was reduced (**Fig. 2d**,**e**), indicating a global shift from the H3-K4me2 to the H3-K4me3 modification state, while H3-K4me1 levels remained constant (**Fig. 2d**). Together, these data indicate that H3-K4me3 is preferentially demethylated by Jhd2p *in vivo* and that Jhd2p contributes to global regulation of H3-K4me3 levels. Despite global changes in H3-K4 methylation, Jhd2p deletion strains are viable and healthy. To try to uncover functional defects in the Jhd2p deletion strain, we examined the strain's phenotypes under a number of conditions (**Supplementary Table 1** online). This analysis revealed no phenotypic defects in the Jhd2p deletion strain, with the exception of a subtle enhancement

of telomeric silencing (**Fig. 2f**). Therefore, disruption of Jhd2p and overexpression of Jhd2p have opposite affects on telomeric silencing. Although the possibility cannot be ruled out that the observed phenotypes are due to demethylation of nonhistone substrates, these observations presumably demonstrate a role for Jhd2p and H3-K4 methylation in regulation of telomeric chromatin function.

The identification of histone demethylases has revealed that histone methylation can be dynamically regulated, like histone acetylation or phosphorylation[10,11,20]. Here we demonstrate that Jhd2p dynamically regulates H3-K4 methylation in budding yeast. Jhd2p preferentially demethylates the H3-K4me3 modification state *in vivo* and contributes to regulation of telomeric silencing. Notably, none of the JmjC domain–containing proteins in budding yeast can remove H3-K79 methylation, suggesting that this modification may be enzymatically irreversible or that an uncharacterized class of demethylase enzyme with unique enzymatic properties remains to be identified. Further analysis of the dynamics of H3-K79 methylation in budding yeast will be important in determining whether this modification can be dynamically regulated and will help to identify enzymes that could remove this histone modification.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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AUTHOR CONTRIBUTIONS

G.L., R.J.K., K.E.G. and Y.Z. designed the experiments. G.L., R.J.K. and K.E.G. carried out the experiments. R.J.K. and Y.Z. wrote the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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