Defining the function for yeast SMYD lysine methyltransferase

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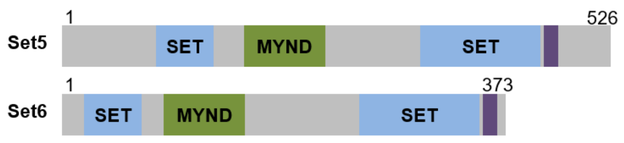
The budding yeast SMYD methyltransferases: Set5 and Set6



Split SET domain: catalytic lysine methyltransferase domain
MYND domain: C2H2 zinc finger, mediates protein-protein or possibly protein-DNA interactions

Mammalian SMYD proteins:

SMYD1	SMYD2	SMYD3	SMYD4	SMYD5
H3K4	Hsp90 Rb P53 MAPKAPK3	H4K5me1 MAP3K2		H4K20(?)

Development: skeletal and cardiac development, muscle cell differentiation
Oncogenesis: (SMYD2, SMYD3)- pancreatic ductal adenocarcinoma, leukemogenesis

- Budding yeast enzymes Set5 and Set6 carry the same domain structure as the mammalian SMYD enzymes and are thought to be ancestral members of this protein family. We previously identified Set5 as an H4 lysine 5, 8 and 12 methyltransferase and determined that its function partially overlaps with the H3 lysine 4 methyltransferase Set1 in repressing genes near telomeres and transposable elements(Green et al,2012; Martin et al,2014; Jezek et al,2017).

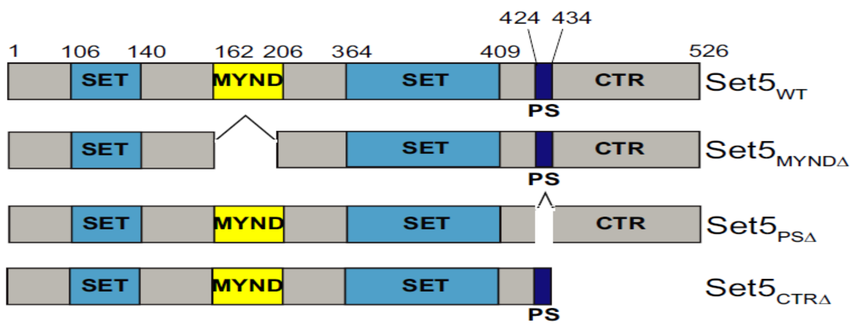


Figure 1. Domain architecture of wild-type Set5 and deletion constructs used for expression in yeast. Set5 contains a split SET domain (blue), a zinc finger MYND domain (yellow), and a post-SET (PS) domain (purple). The extended C-terminal region is designated CTR.

The MYND domain promotes Set5 chromatin association

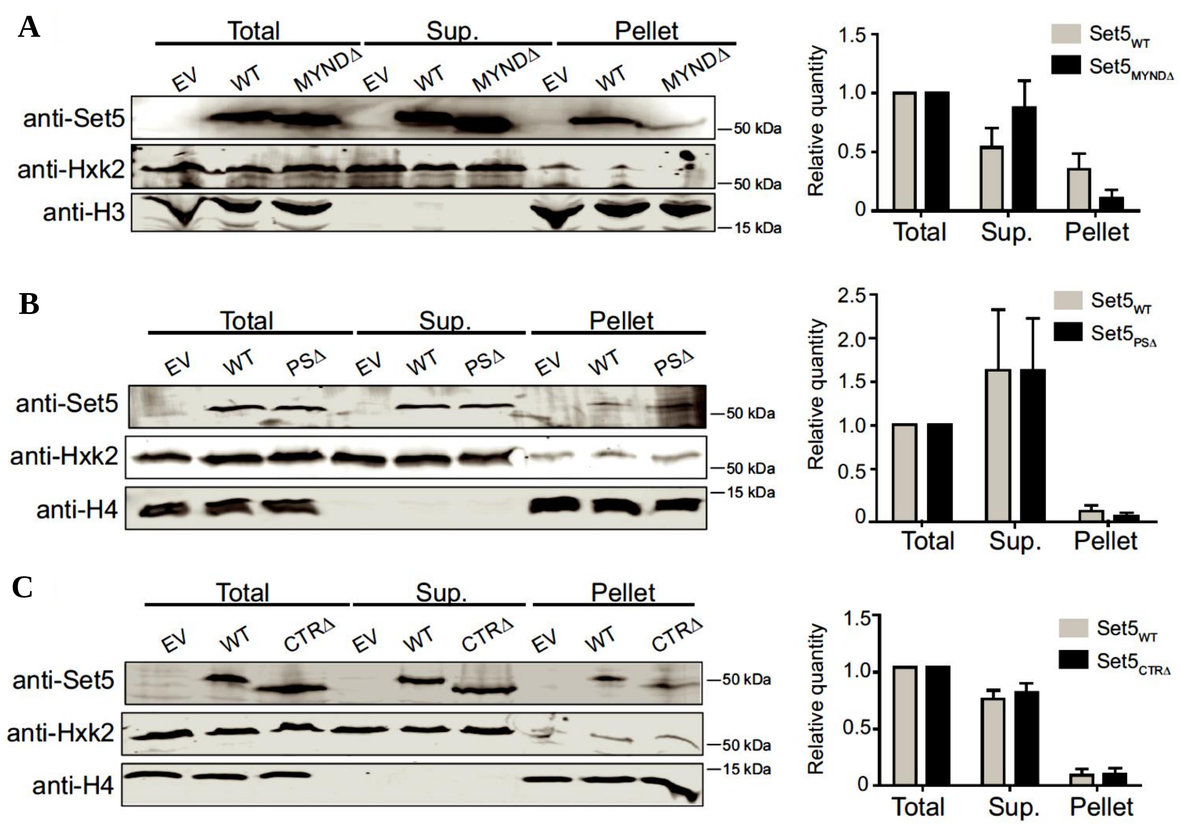


Figure 2: (A) Immunoblot of whole-cell extracts probed with anti-Set5 showing levels of WT and (**A**) $Set5_{MYNDΔ}$, (**B**) $Set5_{PSΔ}$ and (**C**) $Set5_{CTRΔ}$ expressed from vector with an endogenous *SET5* promoter in *set5*Δ yeast cell. (EV, empty vector). Anti-Hxk2 (soluble) and or anti-H3 (chromatin) is shown as a loading control. **(Right)** Quantitation of the relative amount of Set5 in the supernatant and pellet fractions from three independent experiments. Error bars represent the standard errors of the mean (SEM). Quantitation is from three independent experiments.

Yeast Set5 and human SMYD3 interact with DNA via their MYND domains

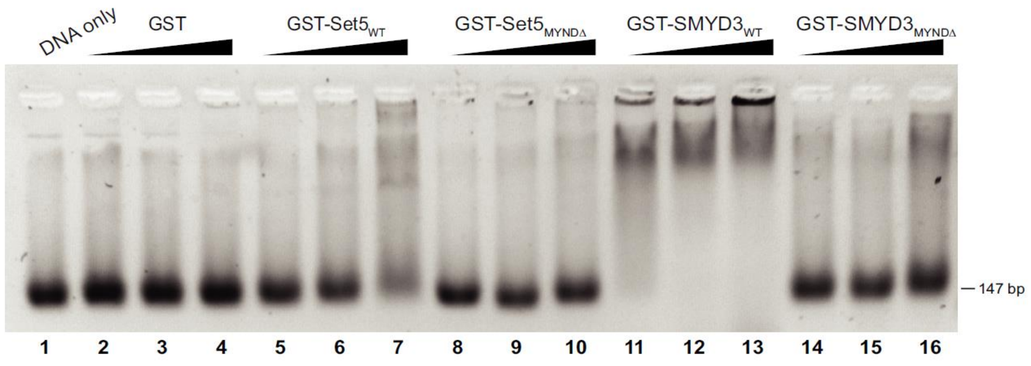
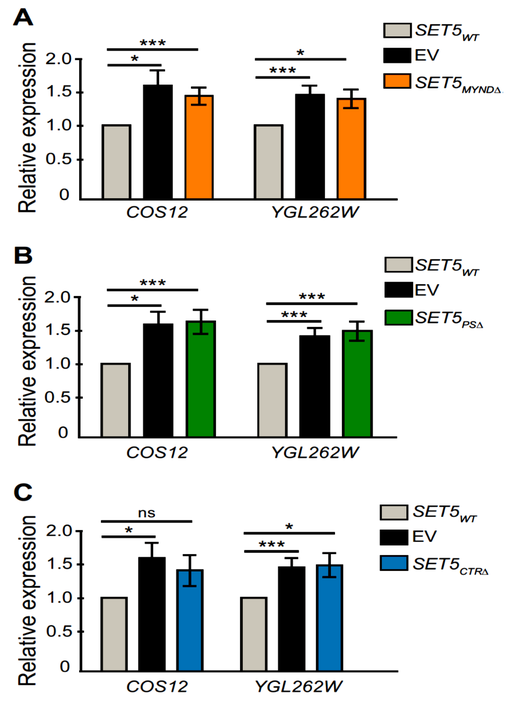


Figure 3: Agarose gel of electrophoretic mobility shift assay (EMSA) using 1.5 pmol of Widom 601 DNA and purified GST, $GST\text{-}Set5_{WT}$, $GST\text{-}Set5_{MYNDΔ}$, $GST\text{-}SMYD3_{WT}$, and $GST\text{-}SMYD3_{MYNDΔ}$. For all proteins tested, 50, 100, and 250 pmol of protein were incubated with the DNA.

Set5-dependent repression of sub telomeric genes



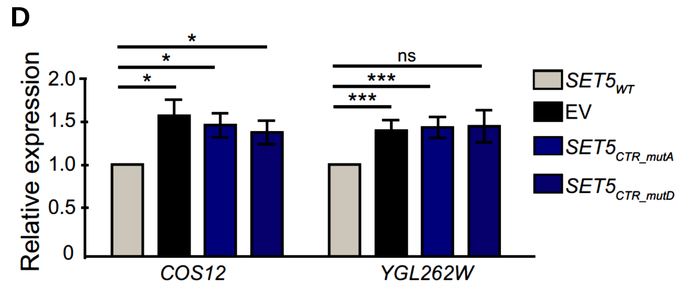


Figure 4: Set5-mediated subtelomeric gene repression requires the MYND domain, post-SET, and CTR. Quantitative reverse transcription-PCR (RT-PCR) of mRNA levels of subtelomeric genes *COS12* and *YGL262W* in *set1 set5* cells carrying either an empty pRS316 vector (EV) or a vector expressing $SET5_{WT}$ or (A) $SET5_{MYNDΔ}$, (B) $SET5_{PSΔ}$, (C) $SET5_{CTRΔ}$ or (D) SET5CTR_mutA, or SET5CTR_mutD. Gene expression relative to *set1 set5* cells expressing $SET5_{WT}$ was calculated for the empty vector and *SET5* mutant cells. The housekeeping gene *TFC1* was used for normalization.

Set5 proteomics: post-translational modifications

Protein region	Site
N-terminal region	S26, T90, T94, S96
Inter-MYND and SET	S301, T305
C-terminal region (CTR)	S458, S461, S462, S466, S469, S470, S475, S476, T504, T511, S512, S517, S520

Table 1: Phosphorylation sites on Set5 identified by mass spectrometry and the regions within the Set5 protein in which these sites were found is indicated.

Phosphorylation within the CTR modulates Set5 enzymatic activity and chromatin association

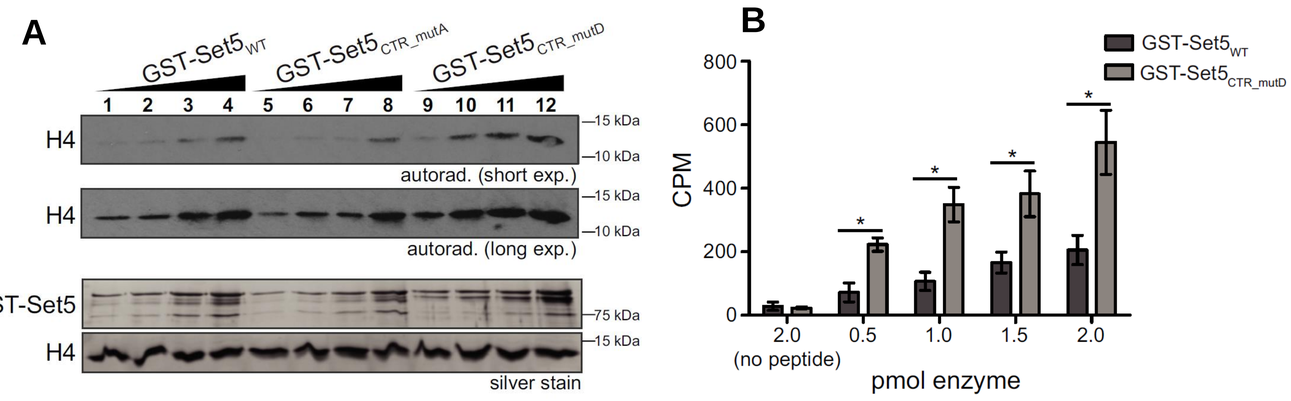


Figure 5. (A) Methylation assay performed using $GST\text{-}Set5_{WT}$, $GST\text{-}Set5_{CTR\_mutA}$, and $GST\text{-}Set5_{CTR\_mutD}$ as enzymes. Short (4 h) and long (24 h) exposures of the autoradiograph are shown. **(B)** Counts per minute (cpm) of tritium-labeled H4 peptide following incubation with either $GST\text{-}Set5_{WT}$ or $GST\text{-}Set5_{CTR\_mutD}$. (C) Subcellular fractionation from set5Δ yeast cells with EV, $SET5_{WT}$, $SET5_{CTR\_mutA}$, or $SET5_{CTR\_mutD}$.

Conclusions and ongoing investigations

- The MYND domain is important for Set5 localization to chromatin. Similar to human SMYD3, Set5 binds DNA in vitro, we speculate that this weak DNA binding, as well as additional protein-protein interactions, work to stabilize the interaction of Set5 with chromatin.
- Phosphoproteomic analysis shows that the CTR of Set5 is highly phosphorylated, and our mutational analysis suggests that phosphorylation within the CTR may enhance methyltransferase activity of the enzyme.
- Set5 works with the H3K4 methyltransferase Set1 to promote repression of telomere-proximal genes. Within Set5, the MYND domain, post-SET domain, CTR and its phosphorylation all act as regulators of either Set5 localization or catalytic activity to influence gene telomeric gene expression.

References and Acknowledgements

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