Histone Lysine Demethylases (2004-present).
Based on the prediction that histone lysine demethylases may contain the JmjC domain, we examined the methylation patterns of five knockout strains (*ecm5*Δ, *gis1*Δ, *rph1*Δ, *jhd1*Δ, and *jhd2*Δ (*yjr119c*Δ)) of *Sacchromyces cerevisiae*. Mass spectrometry (MS) analyses of histone H3 showed increased modifications in all mutants except *ecm5*Δ. High-resolution MS was used to unequivocally differentiate trimethylation from acetylation in various tryptic fragments. The relative abundance of specific fragments indicated that histone K36me3 and K4me3 accumulate in *rph1*Δ and *jhd2*Δ strains, respectively, while both histone K36me2 and K36me accumulate in *gis1*Δ and *jhd1*Δ strains. Analyses performed with strains overexpressing the JmjC proteins yielded changes in methylation patterns that were the reverse of those obtained in the complementary knockout strains. *In vitro* enzymatic assays confirmed that the JmjC domain of Rph1 specifically demethylates K36me3 primarily and K36me2 secondarily. Overexpression of *RPH1* generated a growth defect in response to UV-irradiation. The demethylase activity of Rph1 is responsible for the phenotype. Collectively, in addition to Jhd1, our results identified three novel JmjC domain containing histone demethylases and their sites of action in budding yeast *S. cerevisiae*. Furthermore, the methodology described here will be useful for identifying histone demethylases and their target sites in other organisms. (Collaborators: M. Freitas, CL Liao, Sunny Lo)

Figure 2

A B

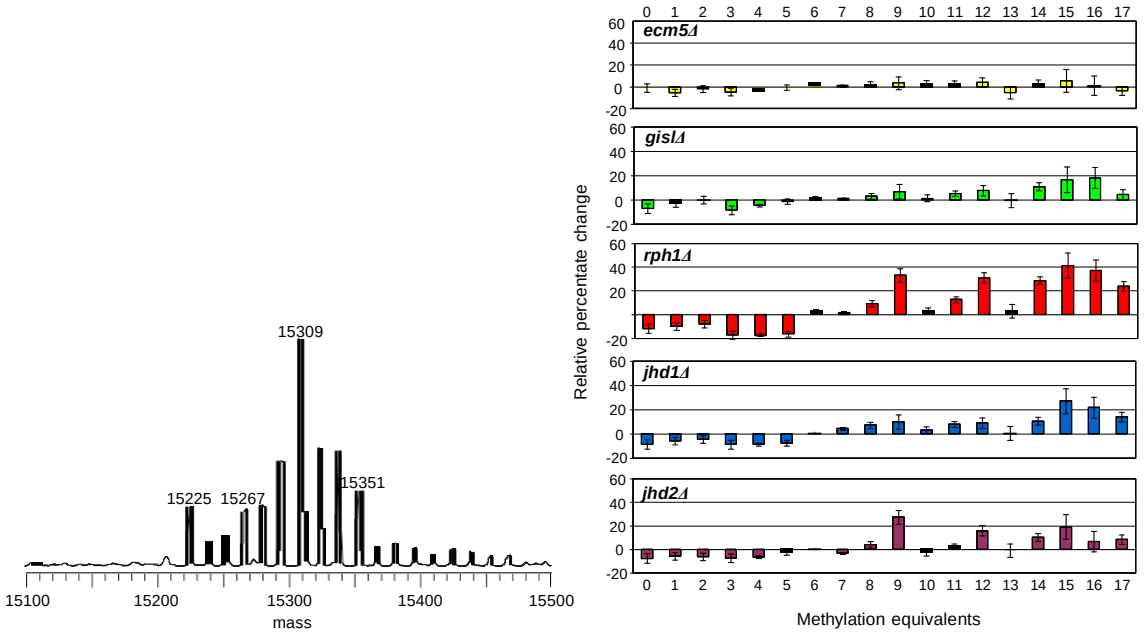

Figure. Global changes of Histone H3 Methylation in JmjC knockout strains. A: A typical LC-MS spectrum of WT H3. The highest peak has six methylation equivalents. B: Bar plots showing "relative percentage change" for each peak in knockout strains relative to WT.

We are also studying RBP2, a human histone lysine demethylase. The human tumor suppressor RB (retinoblastoma protein) interacting protein RBP2 belongs to the JARID1 family of histone H3K4 demethylases. It participates in cell differentiation and is a candidate trxG protein which regulates homeotic gene expression. RBP2 regulates transcription in conjunction with DNA-binding transcription factors and chromatin associated complexes. Importantly, RBP2 also contains an intrinsic DNA binding domain ARID (AT-rich interaction domain). ARID-containing proteins participate in a variety of regulatory processes, including embryonic development and tissue-specific gene regulation. Our recent NSMB paper addresses a novel regulation and targeting mechanism of H3K4 demethylase RBP2. The key results and significance are summarized below: (1) Histone demethylases are the most actively investigated subject and new biological functions are being uncovered daily. We now report the first structural and functional characterization of a DNA-binding domain associated with a histone demethylase. (2) Using SELEX and sequence analyses, we have identified the binding sequence of ARID domain as CCGCCC for the first time, suggesting that ARID domains have different recognition modes (AT-rich, non-specific, and GC rich). (3) Detailed NMR structure and binding studies led us to identify critical residues in ARID for DNA binding specificity. (4) Current studies on histone demthylases focus on identification of novel enzymes. Here, we reported one of the first regulation mechanisms of JmjC demethylases. RBP2 ARID DNA binding is critical in gene-specific transcription. In summary, our studies provide insights in demethylases regulation and are of broad interest in epigenetics and transcription regulation. (Collaborators: Ying-Ta Wu and Li-Jung Juan.)

1. "Identification of Histone Demethylases from Sacchromyces cerevisiae". Shengjiang Tu, Esther M. M. Bulloch, Lanhao Yang, Chen Ren, Pang-Hung Hsu, Wei-Chieh Huang, Chein-Hung Chen, Chung-Lin Liao, Hui-Ming Yu, Wan-Sheng Lo, Michael A. Freitas, Ming-Daw Tsai, J. Biol. Chem. 282, 14262-14271 (2007).

2. "The ARID domain of the H3K4 demethylase RBP2 binds to a DNA CCGCCC motif". Shengjiang Tu, Yu-Ching Teng, Chunhua Yuan, Ying-Ta Wu, Meng-Yu Chan, An-Ning Cheng, Po-Hsun Lin, Li-Jung Juan, and Ming-Daw Tsai,*Nature Struc. Mol. Biol. 15, 419-421 (2008).*

3. "Unambiguous Determination of Isobaric Histone Modifications by Reverse-Phase Retention Time and High-Mass Accuracy". Lanhao Yang, Shengjiang Tu, Chen Ren, Esther M. M. Bulloch, Chung-Lin Liao, Ming-Daw Tsai, Michael A. Freitas,*Anal. Biochem. 396,* 13-22 (2010).

4. "The histone H3K36 demethylase Rph1/KDM4 regulates the expression of the photoreactivation gene PHR1". Chung-Yi Liang, Pang-Hung Hsu, Dai-Fang Chou, Chao-Yu Pan, Wei-Chieh Huang, Ming-Daw Tsai, Wan-Sheng Lo,*Nucl. Acids. Res. 39,* 4151-4165 (2011).

5. "Histone Demethylase RBP2 Promotes Lung Tumorigenesis and Cancer Metastasis". Yu-Ching Teng, Cheng-Feng Lee, Ying-Shiuan Li, Yi-Ren Chen, Pei-Wen Hsiao, Meng-Yu Chan, Feng-Mao Lin, Hsien-Da Huang, Yen-Ting Chen, Yung-Ming Jeng, Chih-Hung Hsu, Qin Yan, Ming-Daw Tsai, and Li-Jung Juan, *Cancer Research 73*, 4711-4721 (2013).