Molecular Cell, Volume *32*

Supplemental Data

Heterochromatin Protein 1a Stimulates Histone H3 Lysine 36 Demethylation by the *Drosophila* KDM4A Demethylase

Chia-Hui Lin, Bing Li, Selene Swanson, Ying Zhang, Laurence Florens, Michael P. Washburn, Susan M. Abmayr, and Jerry L. Workman

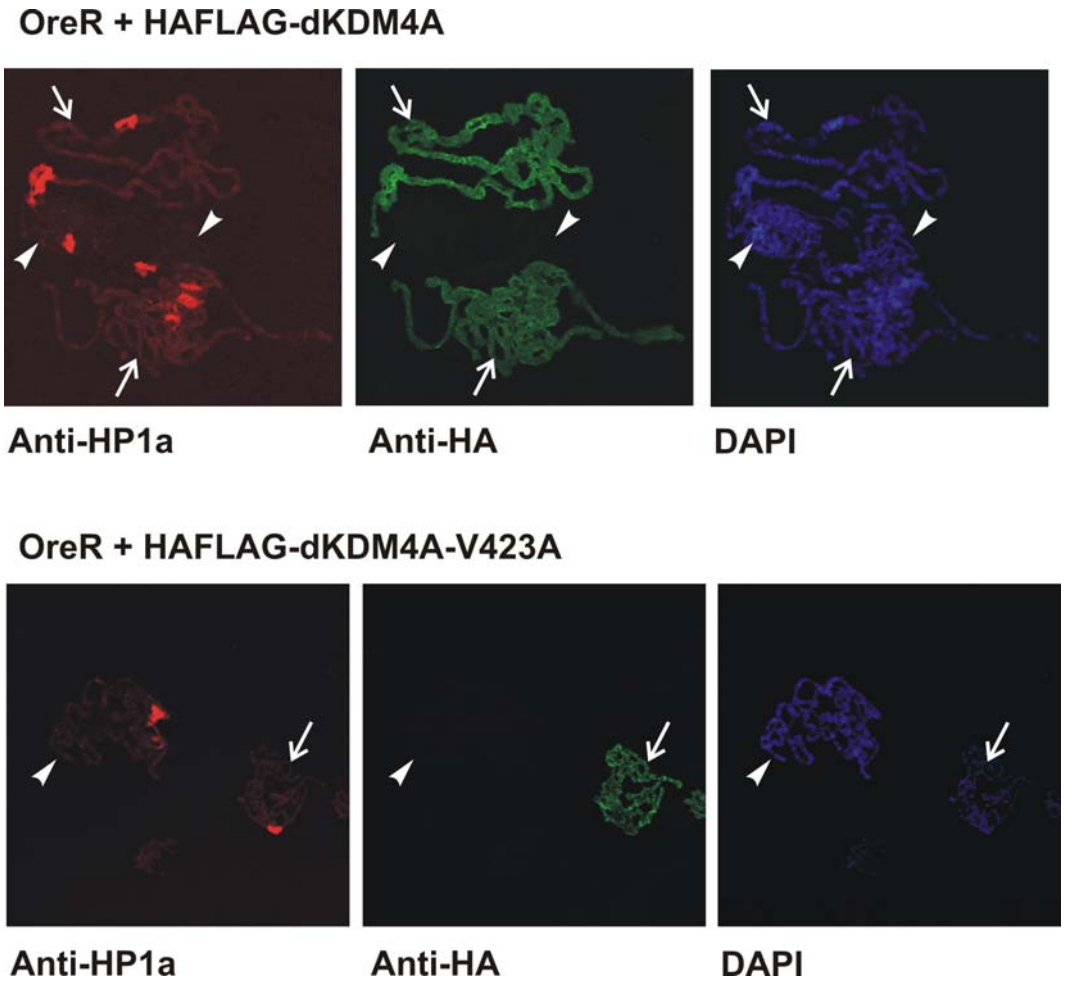

Figure S1. Overexpression of dKDM4A induces HP1a spreading into euchromatin

Salivary glands from wild type and dKDM4A or dKDM4A-V423A-overexperssing larvae were squashed on the same slide. Polytene chromosomes were stained with antibodies against HP1a and HA. The red corresponds to anti-HP1a staining, the green corresponds to anti-HA staining, and the blue corresponds to DAPI staining. Arrowheads indicate polytene chromosomes from wild type larvae, and arrows indicate polytene chromosomes from dKDM4A or dKDM4A-V423A-overexpressing larvae.

Supplemental Experimental Procedures

Plasmids and antibodies

The full length cDNAs of dKDM4A, dKDM4B, HP1a, HP1b and HP1c were cloned into the S2 cell expression vectors pRmHa3-CHA_2FL_2 (Guelman et al., 2006a) or pBacPAK8 containing FLAG or HA tag for overexpression in insect cells. H195A and V423A mutations of dKDM4A and V26M, I191E and W200A mutations of HP1a were generated using the Quik Change II XL Site-Directed Mutagenesis Kit (Stratagene).

Anti-FLAG-HRP antibody (A8592), anti-FLAG M2-agarose (F2426), anti-HA agarose (A2095) were purchased from Sigma. Anti-HA-HRP antibody (12013819001) and anti-HA rat monoclonal antibody (3F10) (1867423) used in immunofluorescence analysis were from Roche. Anti-H3K36me3 (ab9050), anti-H3K36me1 (ab9048), anti-H3K9me3 (ab8898), anti-H3K9me2 (ab1220), anti-H3K9me1 (ab9045), anti-H3K4me2 (ab7766) and anti-histone H3 (ab1791) antibodies were from Abcam. Anti-H3K36me2 antibody (07-369) was from Upstate. Anti-HP1a antibody (C1A9) was from Developmental Studies Hybridoma Bank (DSHB).

Expression and purification of recombinant proteins in Sf21 insect cells

cDNAs of dKDM4A, dKDM4B, HP1a, HP1b, HP1c and derivatives were subcloned into vector pBacPAK8 carrying a N-terminal FLAG or HA tag. Recombinant baculoviruses were generated and manipulated according to manufacture suggestion (BacPAK expression system (Clontech)). Sf21 insect cells were cultured at 27 °C in the Sf-900 II SFM (Invitrogen) supplemented with 10% FBS (SAFC biosciences), and penicillin-streptomycin (Invitrogen). 48 hours after infection, cells were collected and washed with ice-cold PBS, before lysed in 20 ml of ice-cold lysis buffer (50mM HEPES-NaOH (pH 7.9), 500 mM NaCl, 2 mM $MgCl_2$, 0.2% Triton X-100, 10% (v/v) glycerol, 0.5mM EDTA and protease inhibitors). Cell lysates were clarified by ultracentrifugation at 40,000 rpm for 30 min at 4 °C, and were subsequently incubated with anti-FLAG (M2), or anti-HA-agarose beads overnight at 4 °C. The beads were washed three times with lysis buffer, and bound proteins were eluted twice with 1 column volume of elution buffer (0.5 mg/ml triple FLAG or HA peptide in 50mM HEPES-NaOH (pH 7.9), 100 mM NaCl, 2 mM $MgCl_2$, 0.02% NP-40 and 10% (v/v) glycerol).

Immunofluorescence analysis

Stable S2 cell lines expressing HAFLAG-tagged dKDM4A or the mutant dKDM4AH195A were seeded on CultureSlide (BD Bioscience) and induced for 1 day with 100 μM $CuSO_4$. Cells were fixed in 4% paraformaldehyde for 15 min, washed twice with PBS, and permeabilized in 0.5% Triton X-100 in PBS for 5 min. Permeabilized cells were washed with buffer containing 0.1 M Tris-HCl (pH7.5), 150 mM NaCl and 0.05% Tween 20, and blocked in 4% milk in PBS for 30 min. Slides were

incubated with primary antibody overnight at 4 °C using histone methylation specific antibodies at a dilution of 1:500 and anti-HA (3F10) antibody at a dilution of 1:1500. After three times of wash, cells were stained with Cy2 or Cy3-conjugated secondary antibody (Jackson ImmunoResearch) for 1 hour. Cells were washed three times and stained with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) in PBS for 30 min, washed twice with PBS and mounted on glass slides, then visualized by a confocal laser scanning microscope (LSM-510 META, Zeiss).

Purification of dKDM4 from S2 cells and mass spectrometry

Affinity Purification was performed as previously described (Suganuma et al., 2008). Briefly, eight liters of *Drosophila* S2 stable cells were grown and induced with 100 μM $CuSO_4$ for one day. Cells were collected and washed with ice-cold PBS, before lysed with the lysis buffer containing 10 mM HEPES-KOH (pH7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 1% NP-40, 1 mM DTT and 1 mM PMSF. Nnuclei were pelleted by centrifugation at 5,000 rpm for 5 min at 4 °C and extracted using a buffer containing 20 mM HEPES-NaOH (pH7.9), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% (v/v) glycerol, 1 mM DTT and 1 mM PMSF. Nuclear extracts were centrifuged at 14,000 rpm for 15min at 4 °C and then ultracentrafuged at 45,000 rpm for 1.5 hours at 4 °C. The NaCl concentration of the extract was subsequently adjusted to 300 mM. Nuclear extracts were incubated with anti-FLAG (M2) agarose beads (Sigma) overnight at 4°C. The beads were washed three times for 10 min in washing buffer (10 mM HEPES-NaOH (pH 7.9), 300 mM NaCl, 10 mM KCl, 1.5 mM $MgCl_2$, 0.2% Triton X-100, 1 mM PMSF). The complexes were eluted from the beads with elution buffer (0.5 mg/ml triple FLAG

peptide in 10mM HEPES-NaOH (pH 7.9), 100 mM NaCl, 1.5 mM $MgCl_2$, 0.05% Triton X-100 and protease inhibitor). MudPIT analysis of the affinity-purified complexes was carried out as previously described (Guelman et al., 2006b).

RNA purification and RT-PCR

RNA was isolated using Trizol (Invitrogen) and cDNA was generated using SuperscriptIII reverse transcriptase (Invitrogen). Primers for RT-PCR are as follow: dKDM4A F, 5'- TCGAAGCGTTGGATCGAGTA-3'; dKDM4A R, 5'-ACAGGGCAGTTCATTCCATA-3'; rp49 F, 5'-ATGTGTATTCCGACCACGTTACAAGA-3'; rp49 R, 5'-AAGAAGCGCACCAAGCACTTCA-3'.

Primers for RNAi knockdown of dKDM4A in S2 Cells

dKDM4A RNAi F, 5'-TAATACGACTCACTATAGGGAGAtggaatcctcaatcctcgtc-3';

dKDM4A RNAi R, 5'-TAATACGACTCACTATAGGGAGAcaatggatgtgaacgaaacg-3';

LacZ RNAi F, 5'-gcTAATACGACTCACTATAGGCCAAACatgaccatgattacgccaagct-3';

LacZ RNAi R, 5'-gcTAATACGACTCACTATAGGCCAAACgtcccattcgccattcaggc-3'.

Supplemental References

Guelman, S., Suganuma, T., Florens, L., Swanson, S.K., Kiesecker, C.L., Kusch, T., Anderson, S., Yates, J.R., 3rd, Washburn, M.P., Abmayr, S.M., and Workman, J.L. (2006a). Host cell factor and an uncharacterized SANT domain protein are stable components of ATAC, a novel dAda2A/dGcn5-containing histone acetyltransferase complex in Drosophila. Molecular and cellular biology *26*, 871-882.

Guelman, S., Suganuma, T., Florens, L., Weake, V., Swanson, S.K., Washburn, M.P., Abmayr, S.M., and Workman, J.L. (2006b). The essential gene wda encodes a WD40

repeat subunit of Drosophila SAGA required for histone H3 acetylation. Molecular and cellular biology *26*, 7178-7189.

Suganuma, T., Gutierrez, J.L., Li, B., Florens, L., Swanson, S.K., Washburn, M.P., Abmayr, S.M., and Workman, J.L. (2008). ATAC is a double histone acetyltransferase complex that stimulates nucleosome sliding. Nature structural & molecular biology *15*, 364-372.