Supplementary Material

Methylation of H3-Lysine 79 Is Mediated by a New Family of HMTases without a SET Domain

Qin Feng, Hengbin Wang, Huck Hui Ng, Hediye Erdjument-Bromage, Paul Tempst, Kevin Struhl, and Yi Zhang

Supplementary Experimental Procedures

Antibodies, hDOT1L Constructs, and Protein Preparation

The methyl-K79-specific antibody was raised by injection of rabbits with a synthetic peptide coding for amino acids 73–83 of histone H3 with K79 dimethylated (IAQDFmKTDLRF). The methyl-K4, -K9 antibodies were purchased from Upstate Biotechnology. The full-length hDOT1L was derived from two overlapping EST clones: BF507396 and BF982417. The Flag-tagged constructs were cloned into the EcoRI/XhoI sites of a pcDNA-derived vector by PCR. The GST-fusion of the hDOT1L N-terminal fragments were also cloned into the EcoRI/XhoI sites of pGEX-KG vector by PCR. The mutants were generated through PCR-based mutagenesis. All PCR-generated constructs were verified by sequencing. The GST-hDOT1L fusion proteins were purified as previously described [S1]. The recombinant proteins were then cleaved using thrombin following manufacturer's instructions. Proteins were quantitated by Coomassie staining.

Cell Synchronization, Labeling, and Flow Cytometry

To obtain cells synchronized at the G1/S boundary, HeLa cells were treated with 2 mM thymidine (Sigma) for 18 hr, followed by a 9 hr release in thymidine-free medium, and then treated again with 2 mM thymidine for 17 hr to arrest cells at the beginning of S phase. The synchronized cells were released in fresh medium and harvested every 2 hr. HeLa cells synchronized at the mitotic stage were prepared by blocking with 2 mM thymidine for 18 hr, releasing for 3 hr, and then incubating with 100 ng/ml nocodazole for 12 hr. The cells were then washed three times with PBS to eliminate nocodazole before releasing into fresh medium. Cells were harvested every 2 hr, and cell lysates and histones were prepared as previously described [S2]. The cell cycle position of the cells collected at different stages was determined by propidium iodide (PI) staining. For simultaneous measurement of DNA content and levels of specific proteins (Mi2 and mK79), asynchronized HeLa cells were fixed by ice-cold ethanol before treating with 0.25% Triton in PBS for 5 min. Then, the cells were labeled with anti-Mi2 or anti-mK79 antibodies followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories). Finally, the cells were labeled with PI before performing flow cytometry analysis. Flow cytometry was carried out at the Lineberger Comprehensive Cancer Center Flow Cytometry facility.

Supplementary References

S1. Wang, H., Cao, R., Xia, L., Erdjument-Bromage, H., Borchers, C., Tempst, P., and Zhang, Y. (2001). Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. Mol. Cell *8*, 1207–1217.

S2. Wang, H., Huang, Z.Q., Xia, L., Feng, Q., Erdjument-Bromage, H., Strahl, B.D., Briggs, S.D., Allis, C.D., Wong, J., Tempst, P., et al. (2001). Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. Science *293*, 853–857.