SUPPLEMENTARY INFORMATION

Cooperative Action of TIP48 and TIP49 in Histone H2A.Z Exchange Catalyzed by Acetylation of Nucleosomal H2A

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SUPPLEMENTARY MATERIALS AND METHODS

Purification and identification of H2A.Z complexes

The full-length cDNA encoding human H2A.Z was amplified by PCR using primers 5'-ATTGCTAGCATGGCTGGCGGTAA-3' and 5'-TAAGGATCCGACAGTCTTCTGTTG-3' and subcloned into NheI/EcoRI-digested pIRESneo containing FLAG- and HA-tags. HeLa cells were transfected with the resulting plasmid (pFH-H2A.Z-IRESneo) and selected with G418 (500 μg/ml) for 2 weeks. A colony stably expressing ectopic H2A.Z was grown in suspension culture to the density of 1.3×10^6 cells/ml, and nuclear extracts were prepared as described (1,2). For the purification of the initial H2A.Z complex, 300 mg of nuclear extract was passed over phosphocellulose P11 column (Whatman) equilibrated with BC300 and proteins were stepwise eluted with BC300, BC500, BC800, BC1200 and BC1500 buffers. The P11 BC1000 and BC1200 fractions containing expressed H2A.Z were dialyzed against BC300 and applied to anti-FLAG M2 agarose affinity chromatography (Sigma). After extensive washing with BC300 containing 0.2% NP40, the H2A.Z complex was eluted from M2 agarose with BC300 containing FLAG peptide (200 ng/ml, Sigma). For glycerol gradient sedimentation, a portion (0.3 ml) of the initial H2A.Z complex from anti-FLAG immunoaffinity column was loaded onto a 15-40% glycerol gradient (4.7 ml) and spun at 150,000 x g in a Beckman SW Ti55 rotor for 20 h. Approximately 32 fractions (0.15 ml each) were collected from the top of the gradient, and aliquots (16 μl) of the collected fractions were analyzed by immunoblot and silver staining. Antibodies used for western blot analysis were as follows: anti-FLAG and anti-Actin antibodies from Sigma, anti-HA and anti-TRRAP antibodies from Santa Cruz, anti-ANP32E, anti-p400 and anti-H2B antibodies from Abcam, and anti-Tip60 antibody from Upstate. Anti-DMAP1, anti-BAF53 and anti-GAS41 antibodies were kindly gifted by Dr. Robert G. Roeder.

Size Exclusion Chromatography

Nuclear extracts (5 mg) prepared from the H2A.Z cell line were loaded onto the Sephacryl S200 HR column (GE healthcare) equilibrated with the gel filtration buffer (20 mM Tris, pH 7.9, 150 mM NaCl, 0.5 mM EDTA, 0.5 mM EGTA and 10% glycerol). The column was run at a flow rate of 0.2 ml/min and aliquots of collected fractions were analyzed by western blot analysis.

Chromatin immunoprecipitation (ChIP) assays

The 293T cells transfected with TIP49 siRNA or scrambled siRNA were crosslinked with 1% formaldehyde for 10 min at room temperature. Crude cell lysates were sonicated to

an average size of 0.5 kb DNA fragments and subjected to a brief centrifugation to remove cellular debris. The lysates were diluted 1:10 in ChIP dilution buffer (15 mM Tris, pH 8.0, 1 % Triton X-100, 0.01% SDS, 1 mM EDTA, 150 mM NaCl and protease inhibitors), and aliquots of the lysates (1% of total volume) were used for input controls. The chromatin suspension was incubated with salmon sperm DNA/Protein G agarose (Milipore) to remove non-specific background, and the precleared chromatin samples were immunoprecipitated with anti-H2A.Z or rabbit-IgG antibodies. The immunoprecipitated protein-DNA complexes were recovered, washed and incubated overnight at 65°C to reverse the cross-linking. DNA fragments were purified using PCR purification kit (Qiagen), and the enrichment of H2A.Z was analyzed by qPCR with primers specific for 8 regions of the human p21 promoter. Aliquots (1%) of initial sonicated chromatin lysate were used as input controls. The primers (3) used for qPCR are listed in Table S2.

Interaction of TIP48/49 complex with nucleosomes

Nucleosomes were acetylated by the initial H2A.Z complex in HAT reaction buffer containing cold acetyl-CoA (10 μM, Sigma) for 1 h before their immobilization onto Dynabeads. FLAG-TIP48 (75 ng), FLAG-TIP49 (75 ng), or FLAG-TIP48/TIP49 complex (150 ng) were mixed with immobilized mononucleosomes for 80 min at 30°C. The immobilized 207 bp 601 DNA fragments were also included to determine relative DNA binding affinity of TIP48/TIP49. After extensive washing with BC150, the interaction of TIP48/49 with nucleosomes was analyzed by western blot analysis using anti-FLAG (Sigma) antibodies.

SPPLEMENTARY REFERENCES

1. Choi, J., Kim, B., Heo, K., Kim, K., Kim, H., Zhan, Y., Ranish, J.A. and An, W. (2007) Purification and characterization of cellular proteins associated with histone H4 tails. *J. Biol. Chem.*, **282**, 21024-21031.
2. Malik, S. and Roeder, R.G. (2003) Isolation and functional characterization of the TRAP/mediator complex. *Methods Enzymol.*, **364**, 257-284.
3. Saramaki, A., Banwell, C.M., Campbell, M.J. and Carlberg, C. (2006) Regulation of the human p21(waf1/cip1) gene promoter via multiple binding sites for p53 and the vitamin D3 receptor. *Nucleic Acids Res.*, **34**, 543-554.

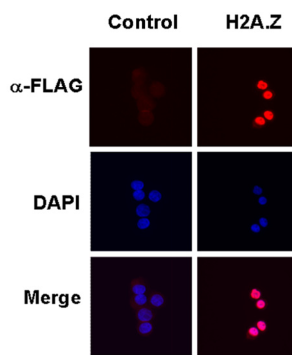

Figure S1. Nuclear localization of ectopic H2A.Z. Regular HeLa cells (left panels) and HeLa-derived H2A.Z expressing cells (right panels) were immunostained with anti-FLAG antibody and countstained with DAPI.

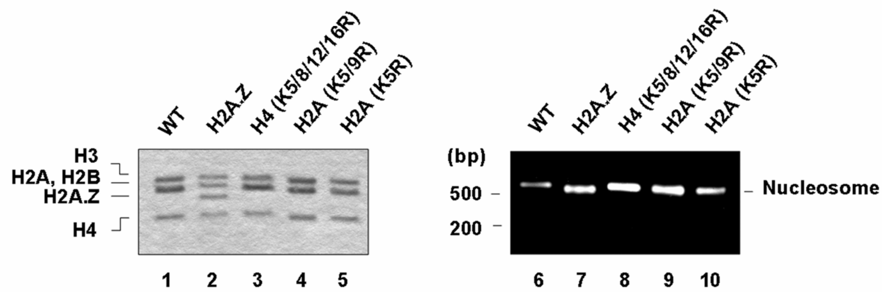

Figure S2. Preparation of recombinant histone octamers and nucleosomes. Recombinant histone octamers containing wild type H2A (lane 1), H2A.Z (lane 2), mutant H4 (lane 3), and mutant H2A (lanes 4 and 5) were prepared as described under "MATERIALS AND METHODS" and analyzed by 15% SDS-PAGE and Coomassie blue staining. Nucleosomes were reconstituted using histone octamers and 5' biotinylated 207 bp DNA fragments containing 601 nucleosome positioning sequence by salt gradient dialysis. Reconstituted nucleosomes were analyzed by 1.5% agarose gel electrophoresis with ethidium bromide staining (lanes 6-10).

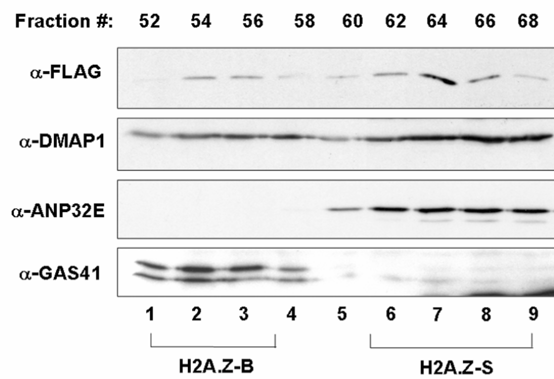

Figure S3. Separartion of small and big complexes by size exclusion chromatography. Nuclear extracts prepared from the H2A.Z cell line were fractionated by Sephacryl S200 HR size exclusion column as described under "supplementary Material and Methods". The fractions were analyzed by western blot analysis using the indicated antibodies.

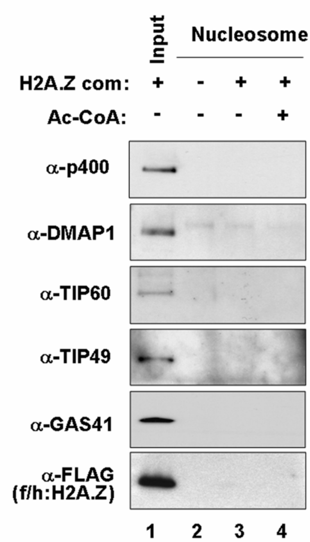

Figure S4. Analysis of purity of acetylated nucleosomes. Acetylated nucleosomes (lane 4) and control unmodified nucleosomes (lanes 2 and 3) were immobilized on streptavidin-conjugated Dynabeads and washed extensively with 150 mM exchange buffer. To confirm the purity of nucleosomes, nucleosomes were separated by 4-20% gradient SDS-PAGE and analyzed by western blotting with the indicated antibodies (lanes 2-4). Lane 1, input of initial H2A.Z complex.

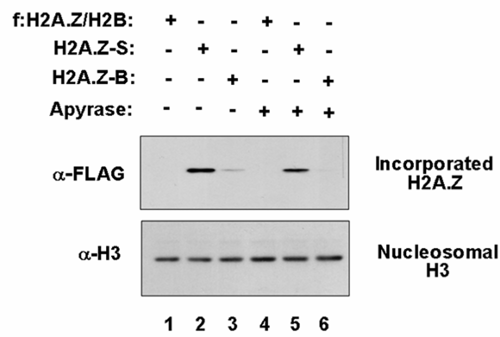

Figure S5. Requirement of ATP hydrolysis for H2A.Z exchange. In vitro exchange reactions were performed as described in Figure 3C (lanes 7-9), but with (lanes 4-6) or without (lanes 1-3) apyrase treatment. H2A.Z incorporation was determined by western blotting of nucleosomes with anti-FLAG antibody (upper panel). Nucleosomal H3 was used as an internal loading control (lower panel).

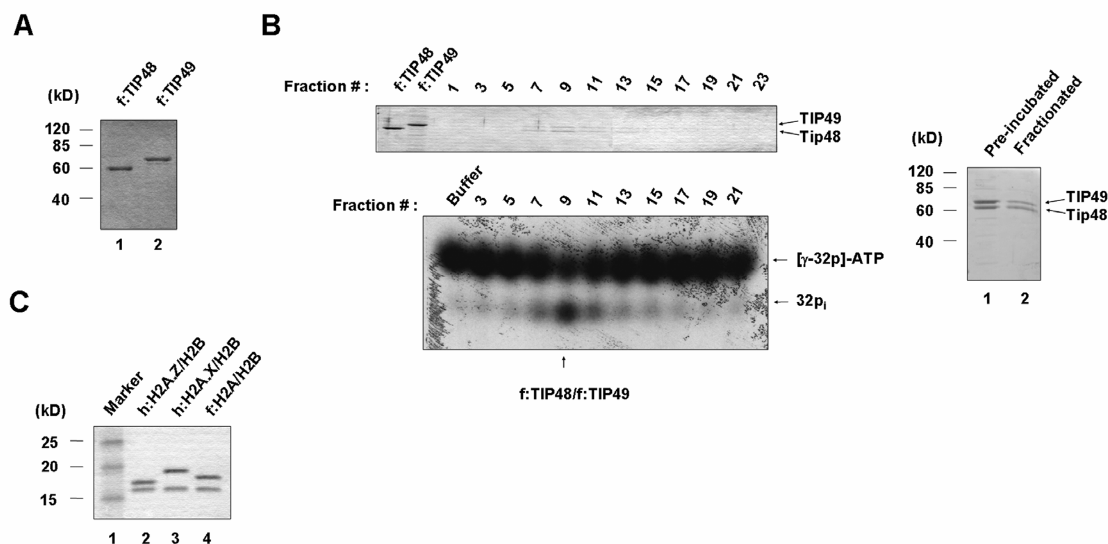

Figure S6. Prepration of recombinant TIP48/TIP49 complex. (**A**) Preparation of recombinant TIP48 and TIP49. FLAG-tagged TIP48 and TIP49 were expressed in bacteria and purified as described in "MATERIALS AND METHODS". The purified proteins were separated on 10% SDS-PAGE and analyzed by Coomassie blue staining. Protein molecular weight markers were indicated. Lane 1, FLAG-TIP48; lane 2, FLAG-TIP49. (**B**) Glycerol density gradient fractionation of TIP48/TIP49 complex. Equal amounts of recombinant f:TIP48 and f:TIP49 were incubated and fractionated by 15-40% glycerol gradient centrifugation as describe in "MATERIALS AND METHODS". Total 24 fractions (200 μl each) were collected from the top to the bottom, and analyzed by 4-20% gradient SDS-PAGE and Coomassie blue staining. After ATPase reaction with aliquots (8 μl) of glycerol density gradient fraction and [γ-^{32}P] ATP, ATP hydrolysis was examined on 12% denaturing polyacrylamide gel (19:1) in a 1 × TBE buffer. Fractions used in ATPase assay were indicated. Glycerol density gradient fraction 9 showing high ATPase activity was concentrated to use in H2A.Z exchange assays. The purity of final TIP48/49 complexes (lane 2) was confirmed on 12% SDS-PAGE followed by Coomassie blue staining. The pre-incubated TIP48/49 (lane 1) was also loaded as a control. (**C**) Preparation of histone dimers. His-H2A.Z-H2B dimer (h:H2A.Z/H2B), His-H2A.X-H2B dimer (h:H2A.X/H2B) and FLAG-H2A-H2B dimer (f:H2A/H2B) were prepared as described in "MATERIALS AND METHODS". The purity of the dimers were confirmed by 15% SDS-PAGE followed by Coomassie blue staining (lanes 1-4).

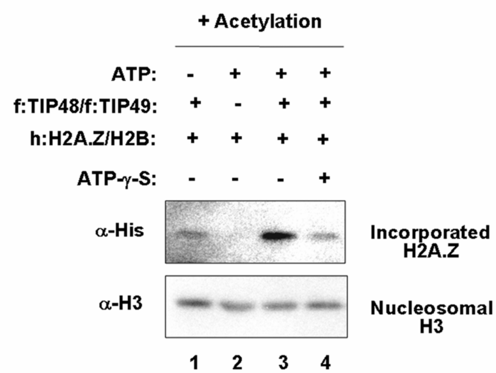

Figure S7. Requirement of ATP hydrolysis for TIP48/TIP49-induced H2A.Z exchange. H2A.Z exchange assays were performed using recombinant TIP48/TIP49 complex as described in Figure 6B, but in the absence (lanes 1-3) or presence (lanes 4) of ATP-γ-S (1 mM). Nucleosomal incorporation of H2A.Z was analyzed by western blot analysis with anti-His antibody (α-His). Equal loading of nucleosomes was confirmed by western blot analysis for nucleosomal H3 (α-H3).

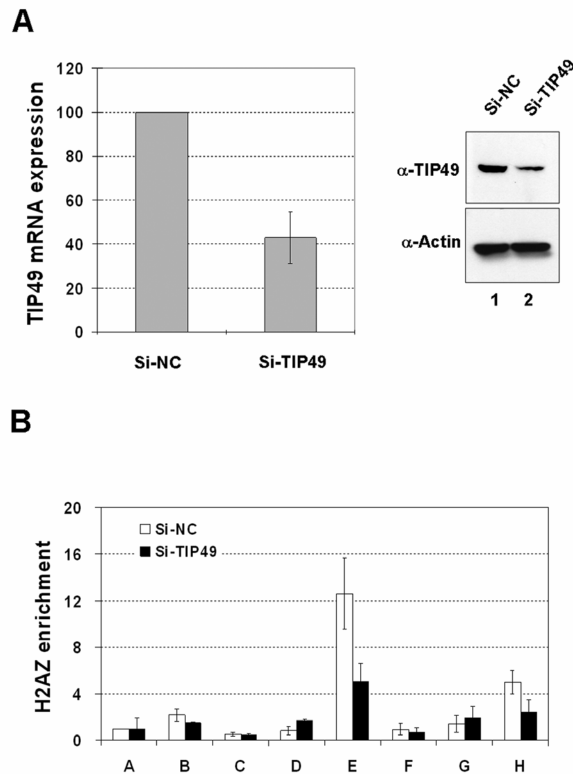

Figure S8. Repressive effect of TIP49 knockdown on H2A.Z exchange at the p21 promoter region. (**A**) Knockdown of TIP49 expression. TIP49 was depleted as described in Figure 7A, but using another validated TIP49 siRNA (Ambion, siRNA ID# 13514) (Si-TIP49). Cells were also transfected with negative control siRNA (Si-NC). Error bars indicate the mean +/- SD of results from three independent experiments. (**B**) TIP48/TIP49-dependent incorporation of H2A.Z at the p21 gene promoter. ChIP analysis of the p21 promoter after TIP49 knockdown was performed as described in Figure 7C. Error bars indicate the mean +/- SD of results from three independent experiments.

Table S1. Primer sequences for RT-PCR

Primer	Strand	Primer sequence (5' → 3')
TIP49	Sense	TGAAGAGCACTACGAAGACGC
	Antisense	ACCTTACTACCCAGCTCCTGA
β-actin	Sense	GTGGGGCGCCCCAGGCACCA
	Antisense	CTCCTTAATGTCACGCACGATTTC

Table S2. Primer sequences for ChIP analysis

Region	Strand	Primer sequences (5' → 3')
A	Sense	CTATGTGCCAAGCTAAGCAC
	Antisense	GGAGTGGAGAAGGAGCAAAG
B	Sense	GCCAGTCATGGTTGTACATGC
	Antisense	CGAGTCTTGCCTGCCTTCAG
C	Sense	CGCGGTGCTTGGTCTCTATG
	Antisense	CCTTTCCCAACAAACAAGGGG
D	Sense	GACATAGCAGGTGTGGTGATGACC
	Antisense	CTGTAGTCCCAGGTATTCAG
E	Sense	CACCACTGAGCCTTCCTCAC
	Antisense	CTGACTCCCAGCACACACTC
F	Sense	GAAATGCCTGAAAGCAGAGG
	Antisense	GCTCAGAGTCTGGAAATCTC
G	Sense	GGAGGCAAAAGTCCTGTGTTC
	Antisense	GGAAGGAGGGAATTGGAGAG
H	Sense	CGTGGGGAAATGTGTCCAGC
	Antisense	GCTCTCTCACCTCCTCTGAG