

Supplemental Data

Histone H3-K56 Acetylation

Is Catalyzed by Histone

Chaperone-Dependent Complexes

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Supplemental Experimental Procedures:

Nucleosomal arrays - Templates containing complete nucleosomes or only (H3/H4)₂ tetramers were generated by salt dialysis as previously described, using chicken histones and a linear DNA fragment containing 11 tandem 5S DNA repeats (Logie and Peterson, 1999). Briefly, 10 µg of DNA templates were mixed with 7.65 µg chicken histone octamers or 6 µg chicken H3/H4 tetramers in 2 M NaCl. Array reconstitution was achieved by stepwise salt dialysis in TE (10 mM Tris pH 8.0, 0.25 mM EDTA) from 2 M NaCl to 2.5 mM NaCl (Logie and Peterson, 1999). The ratio of nucleosomes or H3/H4 tetramers to DNA repeat was sub-stoichiometric (R ~0.8) to ensure negligible free histones in the array reconstitution. Array saturation was monitored by *EcoRI* digestion and analysis on 4% 1x TBE PAGE gels (Logie and Peterson, 1999). Final concentrations of arrays used in acetylation assays were determined by immunoblot comparisons with free H3/H4 tetramers.

Mass spectrometry –

Rtt109/Asf1: 20 pmol of Rtt109, 150 pmol of Asf1, 12.5µM AcCoA, 25µg yeast histone H3/H4 were incubated for 45 min at 30 °C in 500 µl HAT assay buffer with 10mM NaCl, and proteins were then precipitated with trichloroacetic acid. Liquid Chromatography Mass Spectrometry (LC-MS): Histones H3/H4 were separated by reverse phase HPLC (Discovery Bio wide pore 1.0 mm x 150 mm C18 column, 5 µm, 300 Å, Supelco, USA) at a flow rate of 0.05 ml/min over 60 min using 0.1% TFA in H₂O as mobile phase A and 0.1%TFA in acetonitrile as mobile phase B. The flow from the HPLC was coupled to the

electrospray ionization source of a Micromass LCT time-of-flight mass spectrometer (Waters 2690). Tandem mass spectrometry (LC-MS/MS): Histones H3/H4 were digested with trypsin. The digest was desalted by use of a ziptip, redissolved in water and analyzed by nano-LC tandem mass spectrometry on a Shimadzu capillary LC instrument (Columbia, MD) coupled to a ThermoFinnigan LCQ DECA XP+ ion trap (San Jose, CA). Peptides were separated on a reverse phase C18 nano-column (5 cm, 300 Å, 5 µm, I.D. 75 µm) over a 70 min gradient. The mobile phase A was water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid. The data were analyzed by use of an in-house developed database search algorithm, MassMatrix.

Rtt109/Vps75: Enzyme complex (1.15 µM) was incubated with 20 µg (50 µM) of recombinant *Xenopus* histone H3 with 190 µM acetyl-CoA for 10 minutes in 50 mM Tris, pH 7.5 and 1 mM DTT. Trypsin (1 µg) was then added and reacted for 14 hours at 37 °C. Samples were then run on Agilent 1100 LC MS and peptides identified in Mascot. The *Xenopus* histone H3 contains the observed tyrosine at residue 54 rather than the phenylalanine in the yeast H3.

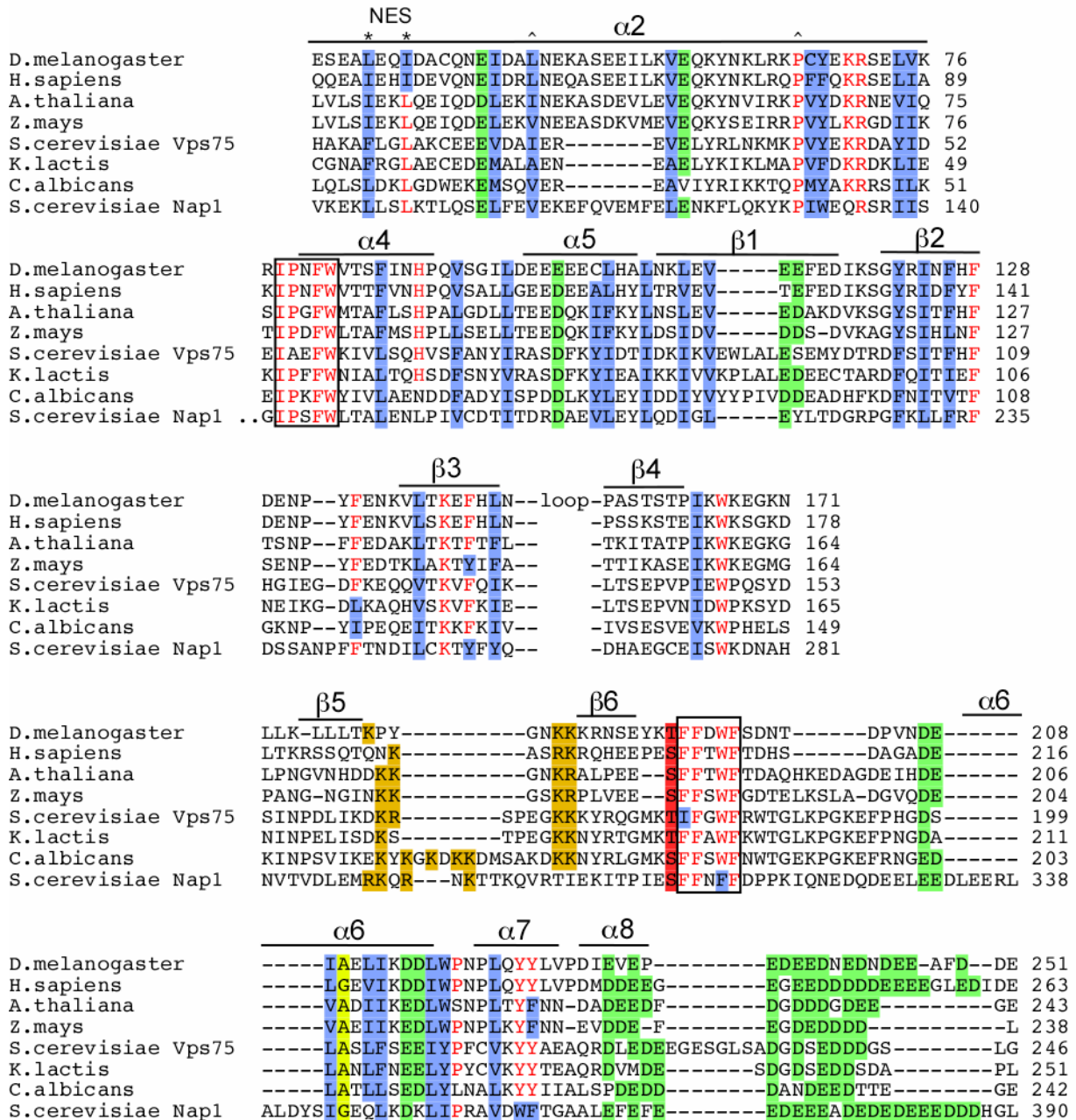


Figure S1. Sequence alignment: Vps75 conservation.

The following protein sequences were aligned using CLUSTAL W (Chenna et al., 2003):

D. melanogaster NAP/SET protein (NCBI Locus AAA74264); *H. sapiens* NAP/SET (NCBI Locus Q01105); *A. thaliana* NAP/SET (NCBI Locus NP_177596); *Z. mays* NAP/SET (NCBI Locus AAK67145); *S. cerevisiae* Vps75 (NCBI Locus NP_014153); *C. albicans* Vps75 (NCBI Locus XP_712165); *K. lactis* Vps75 (NCBI Locus XP_452333); *S. cerevisiae* Nap1 (NCBI Locus P25293). The secondary structure features listed above the sequences were based on the crystal structure of *S. cerevisiae* Nap1 (Park and Luger, 2006); the Vps75 family appears to lack the $\alpha 1$ and $\alpha 3$ helices. Residues identical in most aligned proteins are typed in red. Conserved hydrophobic/aromatic residues are backshaded in blue, conserved acidic residues are backshaded in green, and small residues in yellow. Conserved basic residues in dark orange are found within the region of *S. cerevisiae* Nap1 important for nuclear

localization (Mosammaparast et al., 2005). The two highly conserved, hydrophobic regions of the Nap/Set family important for core packing (Park and Luger, 2006) are boxed. Conserved residues that are essential for nuclear export of yeast Nap1 are marked with an asterisk “*” and the label “NES” (Mosammaparast et al., 2002; Miyaji-Yamaguchi et al., 2003). Conserved residues within the long $\alpha 2$ dimerization helix marked with carets “^” are important for kinks in a long side-by-side helical interaction (Park and Luger, 2006). Not all of the highly acidic C-termini or the non-conserved residues between $\beta 3$ and $\beta 4$ are shown.

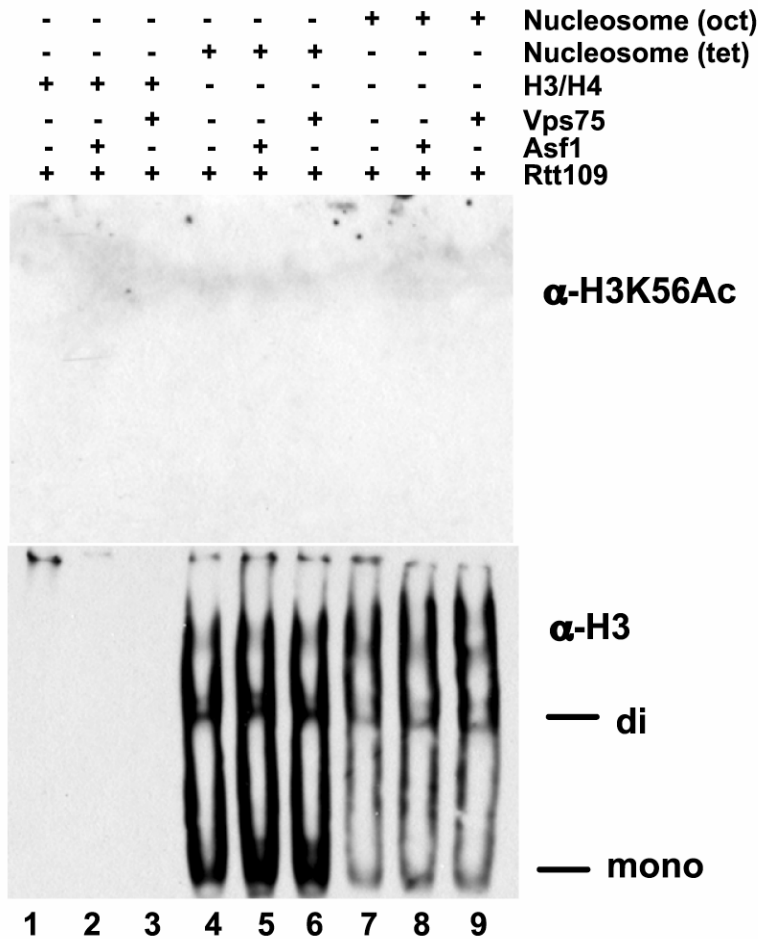


Figure S2. Characterization of histone-DNA complexes. HAT assays were performed with 0.3 pmol of Rtt109 and 3 pmol of Asf1 or Vps75 where indicated. Substrates were 2 pmol of either chicken (H3/H4)₂ tetramers in solution (lanes 1-3), tetramers deposited onto arrays of 5S DNA (lanes 4-6), or complete nucleosomes on 5S DNA arrays (lanes 7-9). Products from the same reactions as shown in Figure 3A were partially digested with *EcoRI*, separated on a 4% native PAGE gel and blotted to a membrane prior to immunodetection. The mono- and di-nucleosomes detected with the anti-H3 antibody are indicated (lower panel, lanes 7-9). A ladder of (H3/H4)₂ multimers are also observed (lower panel, lanes 4-6). In contrast, these species were not recognized by the anti-H3-K56ac antibody (upper panel).

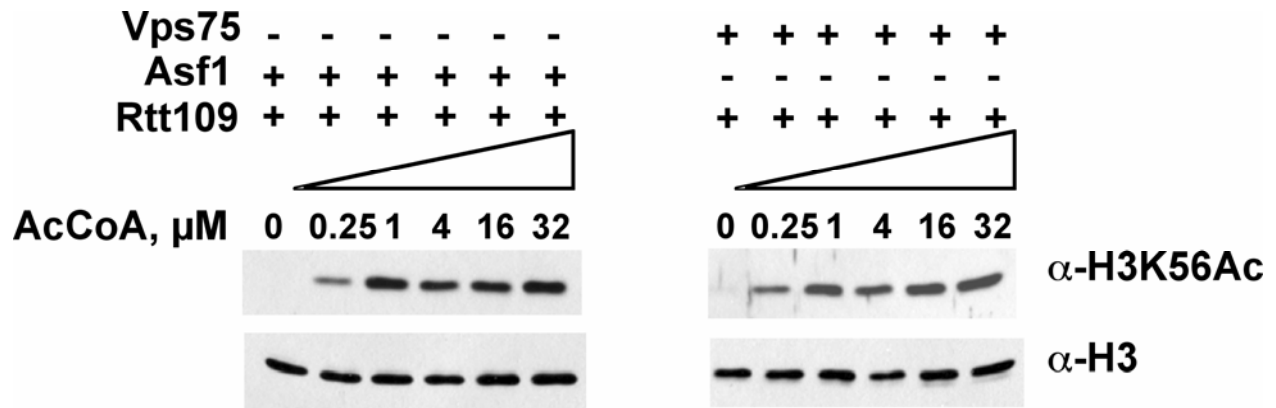


Figure S3. Similar K_m for Ac-CoA for the Rtt109-Asf1 and Rtt109-Vps75 complexes. Ac-CoA was titrated from 0 μ M to 32 μ M in assays containing 0.4 pmol of Rtt109 and 1.5 pmol of Asf1 or Vps75 as indicated. Products were analyzed by immunoblotting.

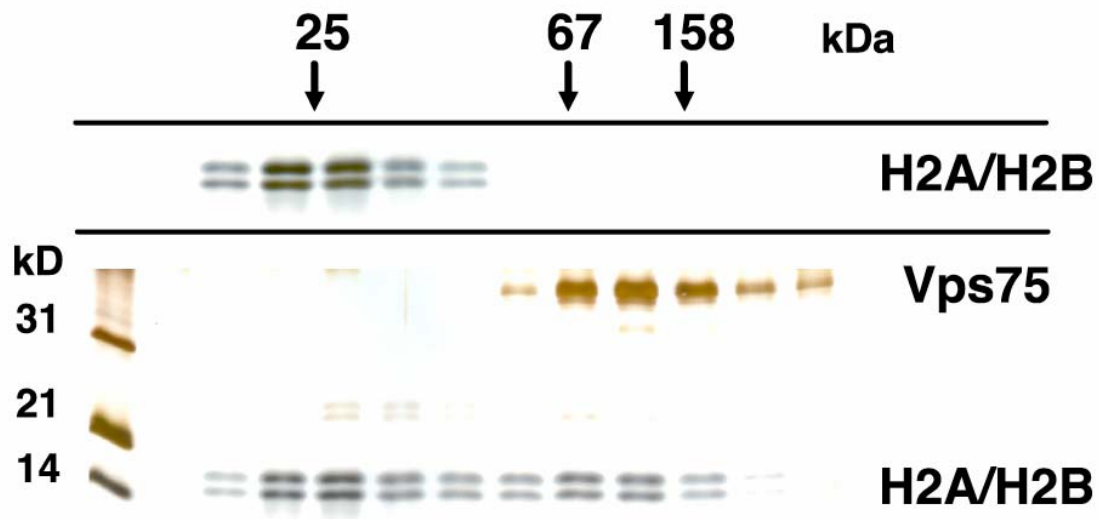


Figure S4. Histone H2A/H2B binding by Vps75. Vps75 and histones H2A and H2B were analyzed by velocity sedimentation on glycerol gradients as described in Figure 2.

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CLUSTAL W (1.83) multiple sequence alignment

Aterreus      YEIGRDSKVLPTAEKVTIRHVSSAPTFCGALFAAPPGEQSESTFCENHFLSVS----AA 355
Coccidiodes  -----QALPKGVKLSVRHVACPTTCEPIFSPFPGEPEPTKCNHFLAVSIRPEEAP 64
Spombe       -----ESILEGRKLSIYHLKSTLEKCFPLFGQS--KSKDFQFGSHLFLVE----- 50
Scerev       -----KVFFSLEVIVYVTLWDEADAERLIFVS--KADTNGYCNTRVSRVDITKILE 109
Umaydis      -----IGALEEQIFITASWSPLTQANAPPDSDARDETEHTDNSPELLILAMELYLYT 115
          : : : . . . . .

Aterreus      SDEHDGAEVIVFGMEVLVYSTHLLTIFVSKADSTGYLHV---LKLPPRASVLRRIISGTF 412
Coccidiodes  TDTDSDVELLILSIEVLVYTRRLATVSVKADSTGYLYL---LNQPRASLIRTRVTFV 121
Spombe       -----EQNVPIFGMECIVYKRNKEIVFVSKADSTGFG-----SKGVSCNSLAFCC 97
Scerev       FILSIDPNYILQVKVPAIRSYKISPELISAASTPARTLR---ILARRLKQSGSTVLKEI 166
Umaydis      IPEFGSAVLVYKLDSSGYAPQS IPTPLRQAVTHVFTHKPKLFGNTLTATLSSAITQHF 175
          : : . . . . .

Aterreus      LS-----YLVQTRQRPVRLVLSLFAQAQNYLPPGSIENPEKHVLDLDRGLIKWVCRAV 466
Coccidiodes  LS-----SLLREYQRPGRIRLVLSLFAQAQNYLPPGSIENPKHVLDLDRGLIKWVCRAV 175
Spombe       VT-----LIDGLRKGGAENVTLTFAIAQGYLFPESVDNGQKHVLDNSGLLRWVWVCL 151
Scerev       ESPRFQDLYLSFTCPREILTKICLFPASQYLPFSSSKNSKHHILNGEELMKWVGFIL 226
Umaydis      TSEFRHWSTQAASAAVNVSHLSVHILARSQRAYLFPSSPENPKVLSDAALIKWVRAVM 235
          : : : . . . . .

Aterreus      DPILREYEPESGSHDK---GAADQAESAASAKSATAFLVPGCDKFETRGGFFPSAKTDD 522
Coccidiodes  DPILRDFEPEKHTTNKALKGEGPDHAEAVNTATAYLIVPGLDQHETRALFFSTARLDF 235
Spombe       EKLRKYTDSEAPNDS-----EKQNSTLLPKAYLVPFVPLEN--IRSYLPN----- 195
Scerev       DRLLIECFQN-----DTQAKLRIPGEDPARVRSYLRG----- 258
Umaydis      SDVVVATREDESPIRA-----ADFKRQLINARAYVYVPGYTKLESHFLVPLARQDSS 287
          : : . . . . .

Aterreus      -----KDRPRFVNSYPVRQLCDNPSA----- 543
Coccidiodes  -----KDKFRWLNSHPLYQVCANFGA----- 256
Spombe       -----RHWIESN-----AITTG----- 207
Scerev       -----MKYPLWQVGDIFT--SKENS----- 276
Umaydis      DSTTQPPSSDQVLRQANWYGHYPYSSNGAHCSSEDLPLPLHWHASSFCLRESDNHVTTV 347
          * .

Aterreus      -----PPRCLIPRFPDDPKTRFLIDLDELPEQTTESSGATEN----- 580
Coccidiodes  -----PPRCLVPRFPDDPKARFLDDLDEEIFGHATTSTRNKTPKCVRDII 301
Spombe       -----KAVEELRFPDDPKCRYLCELQDEKSDMS----- 236
Scerev       -----LAVYNIPLFPDDPKARFIHQLAEDRLLKVS----- 307
Umaydis      QDDPSKDDYKPRVTRVPTLMPHFCDPKTRFLDEMARDAEHSGVKVTTSTASTASTA 407
          : * * * * * : : : :

Aterreus      -----AGQWRSVKSLDQFVEMMSFRQEC SAGRLVGFNLVLPVPPGLVNSVQMT 628
Coccidiodes  DVTEASSQDHAATGRWFSVKSLEEFWEMMTRQEC SAGRLVGFIMVNVNPPGLKSDGLD 361
Spombe       -----VEEFNDTLTYRQECSSGKLVGFFTLQLQ----- 264
Scerev       -----LSSFVWIELQERQEFKLSVTSSVMGISGY----- 335
Umaydis      STTASNGAVDDNVAQAQNGSKDQIDDLQLRQEHSPQDRNTNKRAAKDSDWQDKADKRT 467
          : : : * * *

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Figure S5. Conservation of Rtt109 among fungal species.

The following protein sequences were aligned using CLUSTAL W (Chenna et al., 2003):

Aterreus = *Aspergillus terreus* (NCBI Locus XP_001215675); Coccidioides = *Coccidioides immitis* RS (NCBI Locus EAS31131); Spombe = *Schizosaccharomyces pombe* SPBC342.06c (NCBI Locus CAB46776); Scerev = *S. cerevisiae* Rtt109 (ORF YLL002W); Umaydis = *Ustilago maydis* 521 hypothetical protein UM02284.1 (NCBI Locus XP_758431). The conserved, charged residues that were mutated to alanines are boxed in red (*S. cerevisiae* Rtt109 amino acids DD287-8, and RQE318-320).

Supplemental References:

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