SUPPLEMENTARY DATA

Supplementary Results

Drosophila dRad6 plays a conserved role in histone H2B ubiquitination and histone H3 methylation

Drosophila dRad6/Dhr6 (49) bears the highly conserved ubiquitin-conjugating (UBC) domain typically found in E2 conjugating enzymes (Figure S2A and data not shown). Fluorescence microscopy using either an anti-dRad6 antibody or a DsRed-tagged dRad6 protein suggests that dRad6 is mainly localized in the cytoplasm, with only low-level expression in the nucleus (Figure S2B). Because the loss of yeast Rad6 and its mammalian homolog down-regulates histone modifications, such as H2B monoubiquitination, H3K4 methylation (H3K4me) and H3K79 methylation (H3K79me) (39, S1, S2), we wondered whether *Drosophila* dRad6 plays a similar role in the regulation of histone modifications. To test the effect of dRad6 on H2B monoubiquitination, we transfected Myc-tagged H2B and H2A plasmids together with a His-tagged ubiquitin plasmid into the *Drosophila* embryonic cell line S2 and analyzed their ubiquitination. Control cells were transfected with His-tagged ubiquitin but did not receive Myc-tagged H2B or H2A histones. The involvement of dRad6 in this process was also assessed using S2 cells depleted of dRad6 by RNAi. The results show that H2B, but not H2A, monoubiquitination is dependent on the presence of dRad6 in S2 cells (Figure S2C).

We next examined the effects of dRad6 on histone H3 methylation using dRad6-depleted S2 cells. Trimethylation of H3K4 (H3K4me3) and H3K79 (H3K79me3) was partially dependent on the presence of dRad6, (Figure S2D). In contrast, dimethylation of H3K4 (H3K4me2) and H3K79 (H3K79me2) was not significantly affected by dRad6 depletion. These results suggest that *Drosophila* dRad6 plays a conserved role in the trimethylation of H3K4 and H3K79 but not in the dimethylation of H4K4 and H3K79. Notably, the loss of dRad6 also resulted in a decrease of H3S10 phosphorylation and H3K9 acetylation (Figure S2D). Taken together, these observations suggest that dRad6 plays a conserved role in modulating specific core histone modifications.

Supplementary Figure Legends

Figure S1. dRad6 regulates Dmp53 ubiquitination in Kc cells.

Kc cells transfected with DsRed2 control plasmid or HA-dRad6-DsRed2 plasmid with different amounts, then, treated with DMSO (as a control) or MG132 (25 μM), are indicated. The immunoblot was stained with anti-ubiquitin antibodies to visualize the ubiquitinated form of Dmp53 (upper panel) and monoclonal anti-Dmp53 antibodies to visualize the amount of precipitated Dmp53 as well as the level of Dmp53 ubiquitination (lower panel).

Figure S2. *Drosophila* dRad6 plays a conserved role in the regulation of different histone modifications.

(A) The alignment of *Drosophila melanogaster* dRad6 with yeast Rad6. The protein sequences of *Drosophila* and yeast Rad6 were aligned using DNAman software Conserved sequences are highlighted in black.

(B) dRad6 localization in S2 cells. The top panel shows dRad6 localization in S2 cells transfected with dRad6-DsRed2 plasmid. The bottom panel shows S2 cells immunolabeled with an antibody against dRad6 (anti-dRad6, labeled with Texas-Red). DNA is stained with DAPI (blue). PC, phase contrast.

(C) dRad6 plays a conserved role in H2B monoubiquitination. S2 cells were treated with (+) or without (-) dRad6 dsRNA or GFP control dsRNA prior to be cotransfected with a combination of plasmids encoding His-ubiquitin (His-Ub) and either Myc-H2B (left) or Myc-H2A (right) are indicated. The three panels at the bottom represent cell lysates immunoblotted with different antibodies (mouse anti-Myc to visualize Myc-H2A or Myc-H2B, rabbit anti-dRad6 to visualize dRad6, and mouse anti-tubulin to visualize tubulin), as indicated.

(D) dRad6 is essential for the regulation of H3K4Me3 and H3K79Me3. Western blot analysis shows changes in histone H3 modifications of S2 cells treated with GFP control (Cont) or dRad6 dsRNA (dRad6-RNAi). Equal amounts of proteins from Cont and dRad6-RNAi samples were used for SDS-PAGE is indicated by H3. The specific antibodies against histone H3 modifications are indicated to the right of the gel.

Supplementary Table

Table 1

Primer Name	Sequence

HA-dRad6-DsRed2(+)	ataactagtaccatgtacccatacgatgttccagattacgcttcaacacccgcacgcagacgtcttatgag
HA-dRad6-DsRed2(-)	gccgaattcatcgatgaaactctgctcaac
Myc-H2A(+)	ctggaattctctggacgtggaaaaggtgg
Myc-H2A(-)	gcagcggccgcttaggccttcttctcggtcttc
Myc-H2B(+)	ctggaattccctccgaaaactagtgg
Myc-H2B(-)	gcagcggccgcttatttagagctggtgtacttgg
Ubiqutin-V5/His(+)	gtaaagcttaccatgcagatctttgtgaagactttgac
Ubiqutin-V5/His(-)	gcagcggccgctagcctggattcctccacggagacg
GFP-dsRNA (+)	taatacgactcactatagggatgaagactaatc
GFP-dsRNA (-)	taatacgactcactataggggctcatcatgtttgtatag
dRad6-dsRNA (+)	taatacgactcactatagggtcaacacccgcacgcagac
dRad6-dsRNA (-)	taatacgactcactatagggatcgatgaaactctgctcaac
RT-tubulin (+)	atcggcgctaagttctgg
RT-tubulin (-)	caccgagtaggtgttcatg
RT-Dmp53 (+)	cgcttgatcagatatagccg
RT-Dmp53 (-)	cgcgcagcacggattgctggc
GST-dRad6 (+)	gtcgaattcgtcaacacccgcacgcag
GST-dRad6 (-)	gtcgcggccgcatcgatgaaactctg
GST-Dmp53 (+)	gtcggatcctatatatcacagcc
GST-Dmp53 (-)	gtcaagcttgtggcagctcgtaggcac
Dmp53-GFP (+)	gtcactagtaccatgtatatatcacagc
Dmp53-GFP (-)	gtcgaattcgctggcagctcgtaggcacg
RT-CG4475 (+)	gtggatctggtttacg
RT-CG4475 (-)	ctgcgtgtggatcc
RT-CG11086 (+)	atggccatggagtgtgc
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RT-CG16787 (-)	gtagtgccgtcgcag
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RT-Ku80 (+)	cgtcttcaaggtg

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RT-Eiger (+)	gagttcgacgagttc
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RT-INCENP (-)	ccgcatcttacggttgagtg
RT-Aurora B (+)	tgagatgggcgcccactt
RT-Aurora B (-)	ttcgccacttggtaggtgtact
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RT-Asp (-)	ggtgagaggtgtgaatcgc
RT-Cyc B (+)	cactggaagaaacagccactgggc
RT-Cyc B (-)	ggtggtgggcatcgtagatgtggt
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ChIP-Cyc B (-)	ctcggaagaactggacttgac
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HA-Dmp53 (-)	gtcagatcttggcagctcgtaggcac
HA-Dmp53 1-84(+)	gtcgaaatcggtatatatcacagcc
HA-Dmp53 1-84(-)	gtcagatctgctctagcttgggcagcgtg
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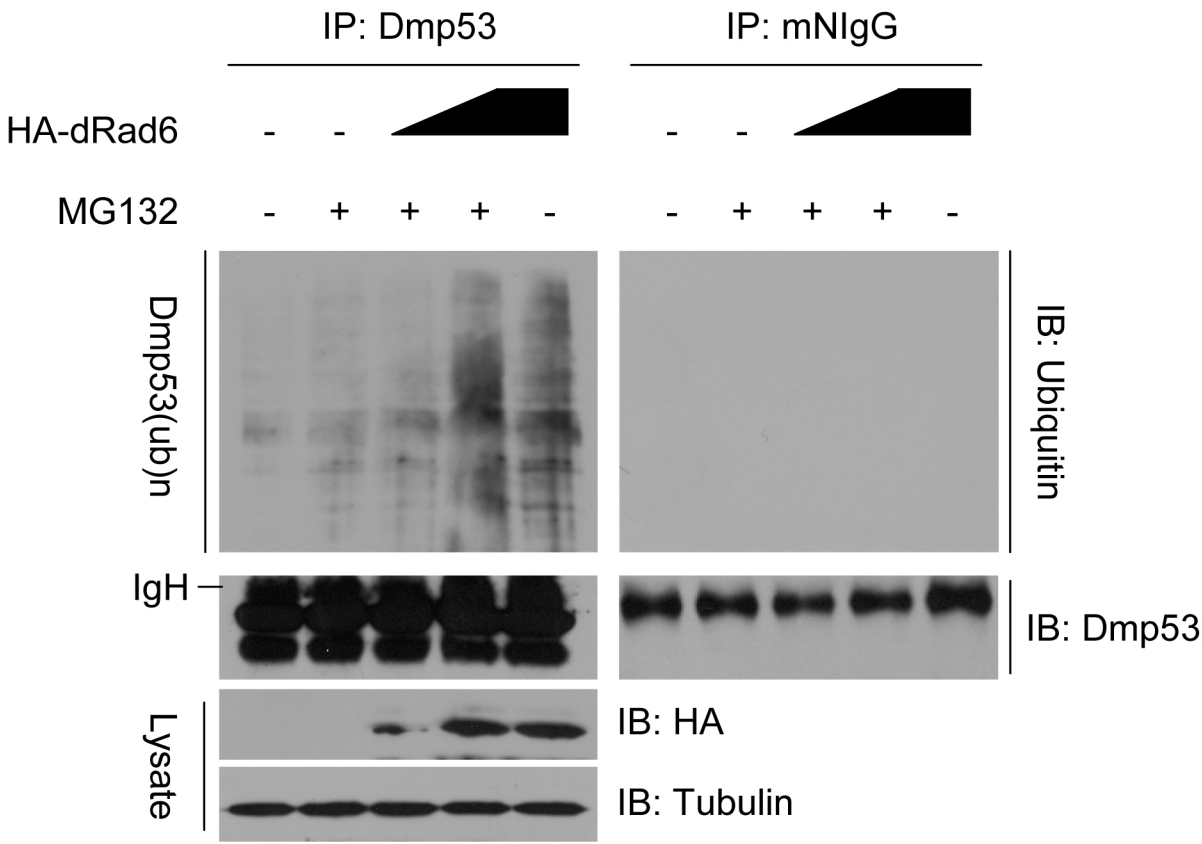
HA-Dmp53 84-282(-)	gtcagatctgggacttgcgcttcttgc

Supplementary Refferences

S1. Robzyk, K., Recht, J., Osley, M.A. (2000) Science 287: 501-504

S2. Dover, J., Schneider, J., Tawiah-Boateng, M.A., Wood, A., Dean, K., Johnston, M., Shilatifard, A. (2002) J. Biol. Chem. 277: 28368-28371

Figure S1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IB: Dmp53
Lysate
IB: HA
IB: Tubulin

Figure S2

A

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Rad6-Yeast  MSTPARRRLMRDFKRMKEDAPPGVSASPLPDNVMVWNAMI  40
dRad6-Dm    MSTPARRRLMRDFKRLQEDPPTGVSGAPTDNNIMIWNAVI  40

Rad6-Yeast  IGPADTPYEDGTFRLLLEFDEEYPNKPPHVKFLSEMFHPN  80
dRad6-Dm    FGPHDTPFEDGTFKLTIEFTEEYPNKPPTVRFVSKVFHPN  80

Rad6-Yeast  VYANGEICLDILQNRWTPTYDVASILTSIQSLFNDPNPAS  120
dRad6-Dm    VYADGGICLDILQNRWSPRYDVSAILTSIQSLLSDPNPNS  120

Rad6-Yeast  PANVEAATLFKDHKSQYVKRVKETVEKSWEDDMDDMDDDD  160
dRad6-Dm    PANSTAAQLYKENRREYEKRVKACVEQSFID---------  151

Rad6-Yeast  DDDDDDDDDEA                               171
dRad6-Dm    -----------                               151
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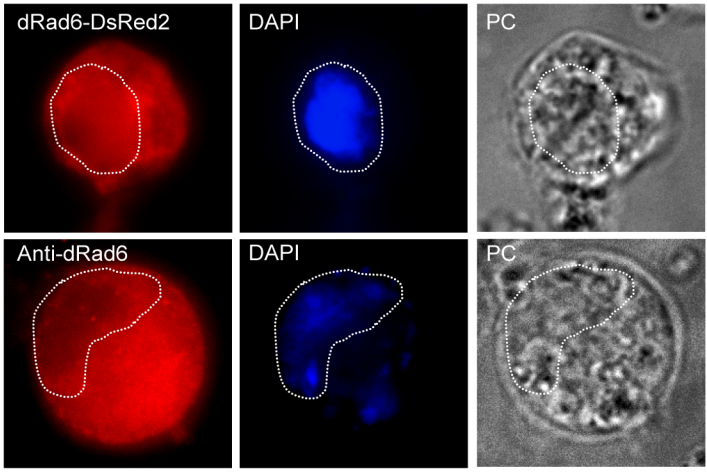
B



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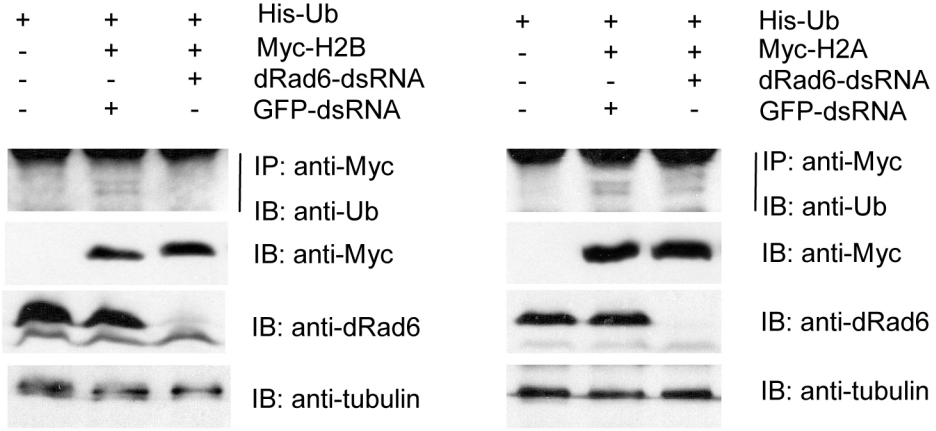

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