Supplemental Information
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Regulation of the Histone H4 Monomethylase PR-Set7 by CRL4^{Cdt2} Mediated PCNA-Dependent Degradation during DNA Damage

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmids

Plasmids for Flag-Cdt2, Myc-DDB1, Flag-Rbx1, and Myc-Cul4A expression were gifts from Dr. Anindya Dutta. p3xFLAG-p21(WT) and p3xFLAG-p21(PIP) were gifts from Dr. Hideo Nishitani. pET28 bacterial expression plasmids for the PR-Set7 PIP mutants and R265G mutant were generated by site-directed and recombinant PCR mutagenesis. Human PR-Set7 wild-type and mutants were subcloned into pMSCV Puro retroviral vector (Invitrogen), pEYFP-C1 vector (Clontech, or a vector in which the EYFP moiety has been replaced by mCherry (Shaner *et al*., 2004)), and p3xFLAG-Myc-CMV-26 vector (Sigma). The siRNA resistant mutation within PR-Set7 was generated by site directed mutagenesis. 53BP1-mCherry plasmid was a gift from Titia de Lange. pMT123 HA-Ub was a gift from Tony Huang. 3xFLAG-tagged PR-Set7 was cloned as an XhoI/BamHI fragment into the pMSCV TtIGPP vector (S. Lowe laboratory, CSHL). iTetONGR* (Anastassiadis et al., 2002) was cloned as an EcoRI/BamHI fragment into pMSCV MB (M. Huebner, CSHL). Retrovirus was produced in 293-GPG cells according to standard methods and used to infect MEFs.

Antibodies and Reagents

An anti-PR-Set7 polyclonal antibody was generated in rabbit. The sources for the following reagents are as follows: FLAG M2 antibody (Sigma), DDB1 antibody (34-2300, Invitrogen), Cdt2 antibody (A300-948A-1, Bethyl), beta-Tubulin (ab6046, Abcam), PCNA (PC10) (sc-56, Santa-Cruz), phospho serine 317 Chk-1 (2344, Cell signaling), phospho-H3S10 (06570, Millipore), phospho-Tyrosine 15 Cdk1 (sc12340, Santa-Cruz), ubiquitin E1 inhibitor UBEI-41 (Biogenova), MMS (Sigma), hydrogen peroxide (Sigma), Adriamycin (Sigma), Hydroxyurea (Sigma), and MG132 (Sigma).

Gene Silencing by siRNA

The siRNA transfections were performed using RNAiMAX (Invitrogen) according to the manufacturer's specifications. The nucleotide sequences of siRNA are given in Supplemental Experimental Procedures. si-oligos for PR-Set7 and si-AllStars as a negative control were purchased from Qiagen. si-oligos for Cdt2 and DDB1 were obtained from Invitrogen.

In Vivo and In Vitro Ubiquitylation Assay

The in vivo experiment was performed in 293T cells transfected with pMT123 HA-Ub for 48 hr and treated with 10 uM MG132 for 4 hr. Cell lysate was prepared as described previously, and immunprecipitated for endogenous PR-Set7 (Abbas et al., 2008). The in vitro assay was done as described previously (Abbas et al., 2008). Briefly, each component of the Cul4/Cdt2 complex was transiently expressed in 293T cells and affinity purified with anti-FLAG antibody. Purified complex was mixed with recombinant PR-Set7 in ubiquitylation buffer. The reactions were incubated at 30 ^{o}C for 1 hr and analyzed by SDS-PAGE and western blotting. UBCH5c was purchased from BostonBiochem.

Protein Interaction Assay and In Vitro DNA Synthesis

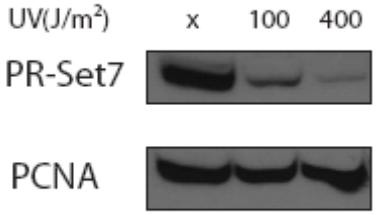
Bacterially expressed Flag-His tagged PR-Set7 WT and mutant proteins were affinity-purified with Ni-NTA agarose (Qiagen) and FLAG M2 agarose resin (Sigma). Purification of HA-tagged PCNA and in vitro DNA polymerization assay were as described (Gibbs et al., 1997).

siRNA Oligos

PR-Set7-7 (target sequence: CTGCAGTCTGAAgAAAGGAAA)
PR-Set7-8 (sense: CCUGUUGAUUGCCAAAAACTTdTdT, antisense:
GUUUUUGGCAAUCAACAGGdTdT)
Cdt2-1 (sense: GAAUUAUACUGCUUAUCGAdTdT, antisense:
UCGAUAAGCAGUAUAAUUCdTdT)
DDB1-1 (sense: ACAGAGUGGCGAGAGCAUUdTdT, antisense:
AAUGCUCUCGCCACUCUGUdTdT)
DDB1-2 (sense: CCUGUUGAUUGCCAAAAACTTdTdT, antisense:
GUUUUUGGCAAUCAACAGGdTdT)

Figure S1. UV-Mediated Degradation of PR-Set7 Occurs at Low Dosages

The protein levels of PR-Set7 decrease as a function of HeLa cell exposure to increased dosages of UV irradiation (100-400 J/m^2), as shown by western blotting. PCNA serves as a loading control.



Supplemental References

Anastassiadis, K., Kim, J., Daigle, N., Sprengel, R., Schöler, H.R., and Stewart, A.F. (2002). A predictable ligand regulated expression strategy for stably integrated transgenes in mammalian cells in culture. Gene 298, 159–172.