Supplementary material

The regulatory functions of H3K36 demethylase Rph1 in the *PHR1* expression

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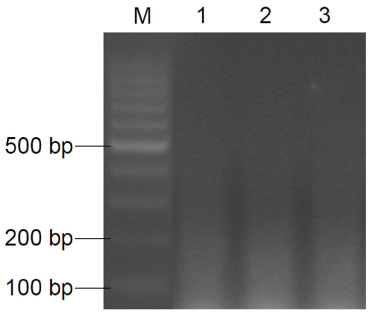
Supplementary table S1

strain	genotype	background	origin
SLY644	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0*	BY4743	(1)
SLY649	*MATα his3Δ1, leu2Δ0, ura3Δ0*	BY4742	(1)
SLY667	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX*	BY4743	(2)
SLY726	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [BG1805]	BY4743	(3)
SLY727	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [BG1805-*RPH1*]	BY4743	(3)
SLY728	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [BG1805-$rph1^{H235A}$]	BY4743	(3)
SLY752	*MATα his3Δ1, leu2Δ0, ura3Δ0, rph1::KanMX* + [BG1805]	BY4742	This study
SLY753	*MATα his3Δ1, leu2Δ0, ura3Δ0, rph1::KanMX* + [BG1805-*RPH1*]	BY4742	This study
SLY794	*MATa ade2-1 can1-100 his3-11 leu2-3 trp1-1 ura3-1 sml1::HIS3*	W303	(4)
SLY795	*MATa ade2-1 can1-100 his3-11 leu2-3 trp1-1 ura3-1 rad53::KanMX sml1::HIS3*	W303	(4)
SLY826	*MATα his3Δ1, leu2Δ0, ura3Δ0, rph1::KanMX*	BY4742	(2)
SLY843	*MATa ade2-1 can1-100 his3-11 leu2-3 trp1-1 ura3-1 rad53::KanMX rph1::KanMX sml1::HIS3* + [pRS425-P_{GAL}] + [BG1805-*RPH1*]	W303	This study
SLY849	*MATa ade2-1 can1-100 his3-11 leu2-3 trp1-1 ura3-1 rad53::KanMX rph1::KanMX sml1::HIS3* + [pRS425-P_{GAL}-*rad53 KD-V5-6XHIS*] + [BG1805-*RPH1*]	W303	This study
SLY851	*MATa ade2-1 can1-100 his3-11 leu2-3 trp1-1 ura3-1 rad53::KanMX rph1::KanMX sml1::HIS3* + [pRS425-P_{GAL}-*RAD53-V5-6XHIS*] + [BG1805-*RPH1*]	W303	This study
SLY864	*MATα his3Δ1, leu2Δ0, ura3Δ0, set2::KanMX*	BY4742	(2)
SLY865	*MATa ade2-1 can1-100 his3-11 leu2-3 trp1-1 ura3-1 rad53::KanMX rph1::KanMX sml1::HIS3* + [pRS425-P_{GAL}] + [BG1805]	W303	This study
SLY867	*MATa ade2-1 can1-100 his3-11 leu2-3 trp1-1 ura3-1 rad53::KanMX rph1::KanMX sml1::HIS3* + [pRS425-P_{GAL}-*rad53 KD-V5-6XHIS*] + [BG1805]	W303	This study
SLY869	*MATa ade2-1 can1-100 his3-11 leu2-3 trp1-1 ura3-1 rad53::KanMX rph1::KanMX sml1::HIS3* + [pRS425-P_{GAL}-*RAD53 -V5-6XHIS*] + [BG1805]	W303	This study
SLY921	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [BG1805-$rph1^{S459A}$]	BY4743	This study
SLY924	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [BG1805-$rph1^{S652A}$]	BY4743	This study
SLY931	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [BG1805-$rph1^{S459AS652A}$]	BY4743	This study
SLY934	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [BG1805-$rph1^{ZF\Delta}$]	BY4743	This study
SLY936	*MATa ade2-1 can1-100 his3-11 leu2-3 trp1-1 ura3-1 sml1::HIS3 dun1::KanMX*	W303	(4)
SLY963	*MATa*, *hhf2-hht2::NAT*, *hta1-htb1::HPH*, *hht1-hhf1::KAN, hta2-htb2::NAT, ura3-52*, *trp1Δ2*, *leu2-3,-112*, *his3-11*, *ade2-1*, *can1-100*, *GAL1-YLR454w*::*TRP1* <pRS315-*HTA1-Flag-HTB1*, *HHT1-HHF1*>	W303	(5)
SLY965	*MATa*, *hhf2-hht2::NAT*, *hta1-htb1::HPH*, *hht1-hhf1::KAN, hta2-htb2::NAT, ura3-52*, *trp1Δ2*, *leu2-3,-112*, *his3-11*, *ade2-1*; *can1-100*, *GAL1-YLR454w*::*TRP1* <pRS315-*HTA1-Flag-HTB1*, *hht1-K4AHHF1*>	W303	(5)
SLY966	*MATa*, *hhf2-hht2::NAT*, *hta1-htb1::HPH*, *hht1-hhf1::KAN, hta2-htb2::NAT, ura3-52*, *trp1Δ2*, *leu2-3,-112*, *his3-11*, *ade2-1*, *can1-100*, *GAL1-YLR454w*::*TRP1* <pRS315-*HTA1-Flag-HTB1*, *hht1-K36AHHF1*>	W303	(5)
SLY967	*MATa*, *hhf2-hht2::NAT*, *hta1-htb1::HPH*, *hht1-hhf1::KAN, hta2-htb2::NAT, ura3-52*, *trp1Δ2*, *leu2-3,-112*, *his3-11*, *ade2-1*, *can1-100*, *GAL1-YLR454w*::*TRP1* <pRS315-*HTA1-Flag-HTB1*, *hht1-K79AHHF1*>	W303	(5)

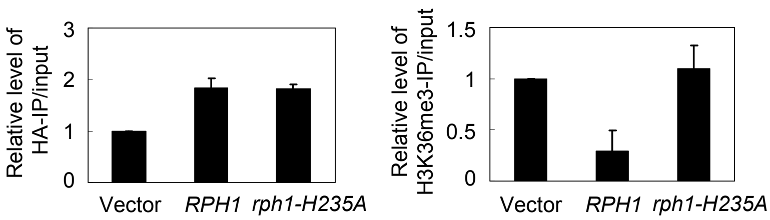
SLY1018	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [pRS425]	BY4743	This study
SLY1020	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [pRS425-*proRPH1-RPH1-FLAG*]	BY4743	This study
SLY1124	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [pRS416-*RPH1pro*]	BY4743	This study
SLY1125	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [pRS416-*RPH1pro-RPH1*-3HA]	BY4743	This study
SLY1165	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [pRS416-*GPD1pro*]	BY4743	This study
SLY1166	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [pRS416-G*PD1pro-RPH1*-6His-3HA]	BY4743	This study
SLY1357	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [pRS416-*RPH1pro*-$rph1^{H235A}$-6His-3HA]	BY4743	This study
SLY1358	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [pRS416-*GPD1pro*-$rph1^{H235A}$-6His-3HA]	BY4743	This study
SLY1159	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [BG1805] + [pRS313-*GPD1pro-RPD3*]	BY4743	This study
SLY1160	*MATa/MATα his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, met15Δ0/MET15, LYS2/lys2Δ0, ura3Δ0/ura3Δ0 rph1::KanMX/rph1::KanMX* + [BG1805-*RPH1*] + [pRS313-*GPD1pro-RPD3*]	BY4743	This study

Supplementary table S2

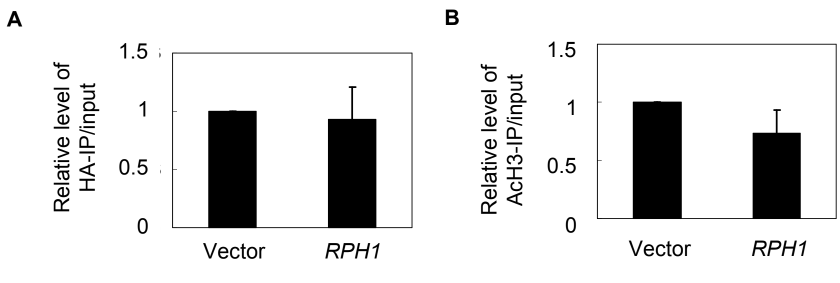
Name	Sequence	Purpose
ACT1-F	TGTCACCAACTGGGACGATA	RT-PCR
ACT1-R	AACCAGCGTAAATTGGAACG	RT-PCR
HUG1-F	ATGACCATGGACCAAGGCCTTAACC	RT-PCR
HUG1-R	TTAGTTGGAAGTATTCTTACCAATG	RT-PCR
U2-F	ATCGATGGGAAGAAATGGTGC	RT-qPCR
U2-R	ACACCTTTCCTTGCAAACCAA	RT-qPCR
PHR1-PF	GAAATCCATAAATCTTTCTATCC	ChIP
PHR1-PR	TGCTGGTTGTCTGTTCTGTGAA	ChIP
PHR1-CF-5'	CGGTAATATCTTCCTCGAACG	ChIP
PHR1-CR-5'	TCATTGATGACATAAACAGCA	ChIP
PHR1-CF-3'	TTGCCAGACGTTTCTGAAGA	ChIP
PHR1-CR-3'	TTTCATAAACCAGCGTTCCC	ChIP
PHR1 UAS-F	TAACAAGCTCCGTCAATTGAACC	ChIP
PHR1 UAS-R	ACTGCTTCCTCGAAAAACGAG	ChIP
PHR1 URS-F	GGGTGAAAGTATGCTTACTTTGAC	ChIP
PHR1 URS-R	ACAATCTCCATTGGTTTAGCCC	ChIP
PHR1 RT3'-F	TCCCGAATTGATTTCTTCCG	RT-qPCR, ChIP
PHR1 RT3'-R	AACTTTCAAAGCACGCTCCC	RT-qPCR, ChIP
ADH1-PF	TTCCTTCCTTCATTCACGCACACT	ChIP
ADH1-PR	GTTGATTGTATGCTTGGTATAGCTTG	ChIP
ADH1-CF	TTCAACCAAGTCGTCAAGTCCATCTCTA	ChIP
ADH1-CR	ATTTGACCCTTTTCCATCTTTTCGTAA	ChIP
ZF del-F	CCGTCCACTCTGGTGAGAAACCTAAGAAAATACCGTGTATTTCAAACG	Site-directed mutagenesis
ZF del-R	CGTTTGAAATACACGGTATTTTCTTAATCTTGTTCTTACCTGATAACAC	Site-directed mutagenesis
S459A-F	CTTTGAAATTAAAAAGAATCTCTGCTTTTCAAGAACAGCCCTTAAAC	Site-directed mutagenesis
S459A-R	GTTTAAGGGCTGTTCTTGAAAAGCAGAGATTCTTTTTAATTTCAAAG	Site-directed mutagenesis
S652A-F	GAATACTCAAAAAGGAAGCTCCTGTCGAGACATC	Site-directed mutagenesis
S652A-R	GATGTCTCGACAGGAGCTTCCTTTTTGAGTATTC	Site-directed mutagenesis



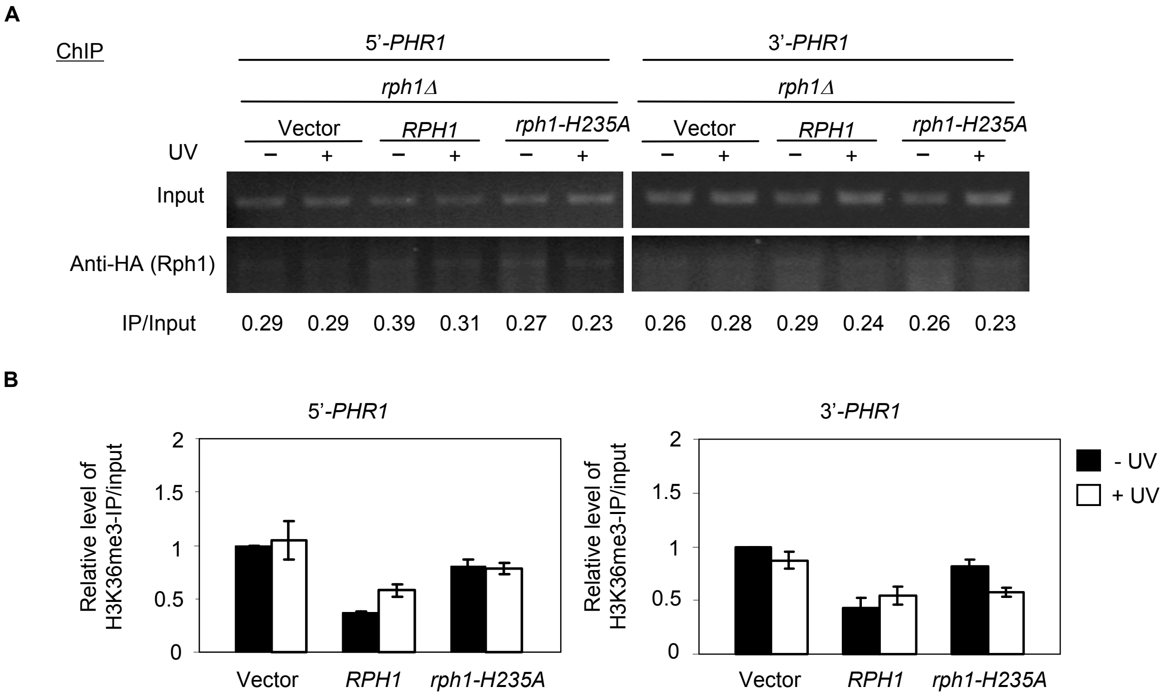
Supplementary figure S1.
DNA fragments were about 100 to 200 bp after sonication for ChIP. 1: Input DNA of *rph1Δ* with control vector. 2: Input DNA of *rph1Δ* with wild-type Rph1. 3: Input DNA of *rph1Δ* with ZF deletion Rph1.



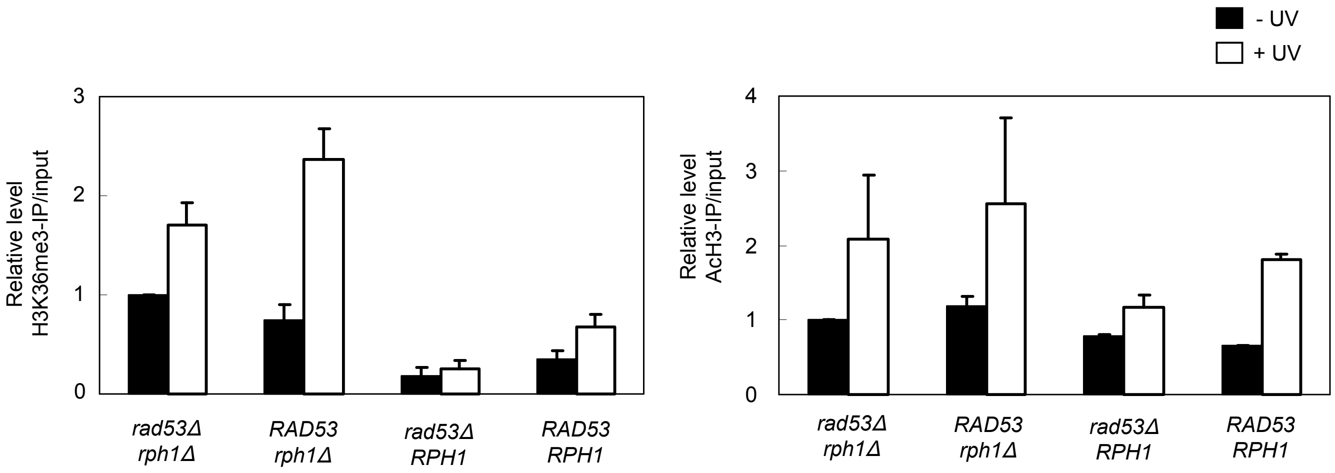
Supplementary figure S2.
ChIP with anti-HA and anti-H3K36 tri-methylation antibodies were performed in *rph1Δ* strains carrying *CEN* plasmid with vector, Rph1 or H235A mutant. DNA abundance was determined by qPCR for URS regions. Error bars represents results from 2 biological repeats.



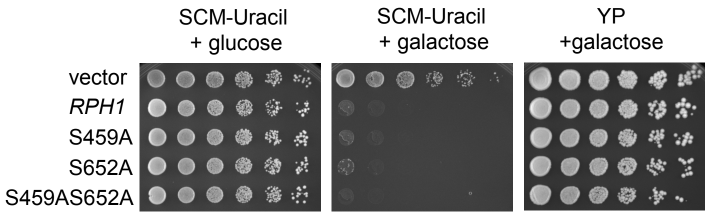
Supplementary figure S3.
No significant association of Rph1 or reduction of H3 acetylation was observed at the *ADH1* promoter. The *rph1Δ* yeast-containing vector and overexpressed wild-type *RPH1* were grown to log-phase and induced with galactose then harvested for ChIP with anti-HA (A) and anti-AcH3 (B). DNA abundance was determined by qPCR for specific *ADH1* promoter. Error bars are the SD of 3 different replicates. .



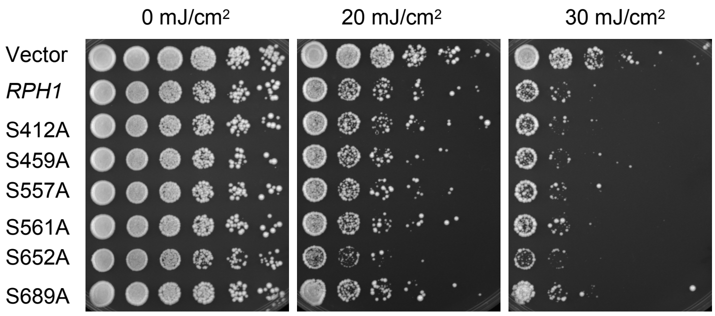
Supplementary figure S4.
No detectable binding of Rph1 was obtained in the 5' or 3' coding regions of *PHR1*. (A) Comparable HA-ChIP samples in Figure 4A were analyzed by PCR specific to 5- or 3-terminal coding regions of *PHR1*. The relative immunoprecipitation (IP)/input values of anti-HA ChIP are shown. (B) Comparable H3K36me3-ChIP samples in Figure 4A were analyzed by PCR specific to 5- or 3-terminal coding regions of *PHR1*. Error bars show the SD of 2 biological replicates.



Supplementary figure S5.
H3K36me3 and acH3 at URS of *PHR1*. Samples are comparable to those in Figure 5D. Bar graphs show qPCR results in URS of *PHR1* promoter. Results are from 2 biological samples.



Supplementary figure S6.
The *rph1*Δ carrying wild-type *RPH1* or mutants under *GAL1* promoter were grown on selective plates with glucose or galactose and YPGal plate after 4-hr induction with galactose . Comparable strains were from Figure 6B. Photographs were taken after 3 days' incubation at 30 ˚C.



Supplementary figure S7.
UV-sensitivity test of 6 phospho-mutants. The *rph1*Δ carrying wild-type *RPH1* or mutants under *GAL1* promoter were grown to early log phase and subject to UV irradiation with indicated doses after 4-hours induction by galactose. All plates were photographed after 3 days' incubation.

Supplementary references

1. Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P. and Boeke, J.D. (1998) Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast*, **14**, 115-132.
2. Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H. *et al.* (1999) Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. *Science*, **285**, 901-906.
3. Tu, S., Bulloch, E.M., Yang, L., Ren, C., Huang, W.C., Hsu, P.H., Chen, C.H., Liao, C.L., Yu, H.M., Lo, W.S. *et al.* (2007) Identification of histone demethylases in Saccharomyces cerevisiae. *J Biol Chem*, **282**, 14262-14271.
4. Zhao, X. and Rothstein, R. (2002) The Dun1 checkpoint kinase phosphorylates and regulates the ribonucleotide reductase inhibitor Sml1. *Proc Natl Acad Sci U S A*, **99**, 3746-3751.
5. Fleming, A.B., Kao, C.F., Hillyer, C., Pikaart, M. and Osley, M.A. (2008) H2B ubiquitylation plays a role in nucleosome dynamics during transcription elongation. *Mol Cell*, **31**, 57-66.