Supplemental Data

Yeast H2A.Z, FACT complex and RSC regulate transcription of tRNA gene through differential dynamics of flanking nucleosomes

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Nucleosome is not positioned on *SUP4* gene

High resolution MNase (micrococcal nuclease) footprinting (Figure S1) shows TFIIIC binding to chromatin but no nucleosomal footprint is seen on the transcribed region of the gene, suggesting unlike *SNR6* (24), gene is not covered by a positioned nucleosome. A chromatin-specific MNase hypersensitivity downstream of box B probably denotes the boundary of a positioned nucleosome downstream, covering the gene terminator.

Swr1 complex is required for H2A.Z deposition in *SUP4* flanking nucleosomes

Similar to other genes having H2A.Z at their ends, a Swi/Snf class ATP-dependent chromatin remodeler, SWR1 complex (58, 59) is found essential for the H2A.Z enrichment on *SUP4* (Figure S2A). Swr1 localizes to the gene region and its occupancy goes up (Figure S2B) after 1 h of repression when H2A.Z levels also show ~2 fold inscrease in both the flanking nucleosomes (Figure 2F).

Nhp6 addition increases the MNase sensitivity of chromatin in vitro

Addition of Nhp6 to *SUP4* naked DNA in vitro is shown to increase the transcription fidelity by improving the TFIIIC interaction with box A, which in turn improves the accuracy of TFIIIB placement upstream (36). Nhp6 is also reported to increase the nuclease sensitivity of the nucleosomal DNA (34). We used IEL technique to monitor the effect of Nhp6 addition on chromatin structure in vitro (Figure S3A). While Nhp6 addition is found to increase the nuclease sensitivity of naked DNA (lanes 5 vs. 6) as well as chromatin (lanes 1 vs. 2); it enhances the protection of nucleosomal DNA upstream of the gene in the presence of TFIIIC, probably by improving the TFIIIC/B-gene interaction (cf. lanes 3 and 4, vertical bar). This may explain the effects of Nhp6 on transcription fidelity seen in the Figure 4. Mapping of MNase cuts shows that in the presence of TFIIIC, nucleosomes in the gene upstream region occupy positions similar to that seen in vivo (Figure 2), further suggesting Nhp6 effects seen in vitro can be extrapolated to in vivo situation. Thus, similar to in vitro observation, Nhp6 could be improving the TFIIIC-nucleosome interaction via altering the conformation of nucleosomal DNA in vivo.

FACT does not regulate the chromatin architecture of SUP4 locus in vivo

Apart from being a part of the FACT complex, Spt16 has a specific role in chromatin alteration on certain pol II-transcribed genes (38). The non-specific, spurious transcription on pol II-transcribed genes is suggested to be the result of a chromatin structure disruption in Spt16 mutant strains (37, 38). Therefore, we probed the effect of yFACT components on the chromatin structure of *SUP4* locus in vivo. The IEL analysis of Nhp6 as well as Spt16 (Figure S3B) and Pob3 (not shown) mutants does not show any change in gross chromatin structure around *SUP4* (Figure S3B, lanes 3-7 vs. lanes 1, 2) at permissive temperature. At non-permissive temperature, the nucleosomal arrays could not be seen on *SUP4* locus as well as TEL VIR region (not shown); suggesting Nhp6, Spt16 and Pob3 do not alter the chromatin structure of *SUP4* locus. Thus, these results suggest that their repressive effect on *SUP4* transcription may be at some other level.

Transcription of CMD1 and U4 genes is independent of Spt16

RNA level of both U4 and *CMD1* in wild type cells (Figure S4, laqnes 1-4) shows a fall under starvation with time, suggesting nutritional stress can repress transcription of these genes. Changes in U4 levels are comparatively lower, probably due to high stability of this RNA. One of these genes, *CMD1* was earlier reported to be Spt16-independent (6). In agreement, RNA levels in the mutant cells remain unaltered in the active state while repression does not show any significant difference from wild type cells.

H2A.Z increase on *SUP4* locus is not due to H3 increase.

Total H3 as well as H2A.Z levels in mutant and wild type cells do not show any significant difference in active state (Figure S5A, cf. lane 1 of each panel). The H3 levels in corresponding *SUP4*-flanking nucleosomes of wild type and mutant cells also match in active state (Figure S5B), suggesting lack of Spt16 activity may be directly responsible for the lower H2A.Z levels in nucleosmes flanking the *SUP4* (Figure 5B). Levels of total H3 (Figure S5A lanes 2-4) as well as levels in *SUP4* flanking nucleosomes (Figure S5B) do not show change even under repression in wild type cells. In agreement with known interaction of Spt16 and H3, a marginal increase in -1 but decreasing levels in the +1 nucleosome can be seen (Figure S5B) in the mutant cells, again suggesting both the nucleosomes follow different dynamics under repression, and may be targets of different chromatin modifiers.

A loss of H2A.Z levels is seen on both the pol II-transcribed genes, *CMD1* and U4 (Figure S5C) but time course of H2A.Z change remains more or less similar in the Spt16 mutant cells. Thus, despite differences in relation of expression level to Spt16, the dynamics of H2A.Z is similar under repression on all three genes, *SUP4* (Figure 5C), *CMD1* and U4 (Figure S5C). Additionally, despite a small loss of H3 in +1 nucleosome (Figure S5B), H2A.Z levels do not change during repression in mutant cells, suggesting H2A.Z does not follow H3 dynamics. Most interestingly, a comparatively late increase in H2A.Z level, 4 hours after repression, is seen when a visible decrease in RNA levels can also be seen (Figure S4), suggesting repression of pol II-transcribed genes in response to nutrient starvation sets in much later than repression of pol III-transcribed genes.

H2A.Z and Spt 16 interact with each other

Truncated Spt16 (60) interacts with Pob3 (Figure S6A, lower panel) but does not associate with H2A.Z (upper panel), suggesting N-terminus of Spt16 interacts with H2A.Z. The results further affirm that Spt16 functions as H2A.Z chaperone in Swr1-mediated H2A.Z dynamics on *SUP4* gene in vivo. In order to follow interaction of the two proteins in vitro, both the proteins were purified to homogeneity (Figure S6B).

Supplementary Materials and Methods

ChIP and Real Time PCR

ChIP was performed according to a rapid protocol as described earlier (17). Cross-linking was done with 1% formaldehyde for 15 mins. in case of histones and 30 mins. for individual FACT subunits. Unless otherwise stated, all normalizations were made against the heterochromatic telomeric region of the sixth chromosome (TelVIR) as the positive control, taking a value of 1 or more as presence and a value less than 1 as absence. As levels of histones on *SUP4* were lower than TelVIR, we also measured them against a hostone-free, mitochondrial gene *COX3* (61) taken as negative control. Primer sequences of amplicons shown in Figure 1C can be found in the Table S1. H3 levels in yeast were estimated using anti-H3 antibody (abcam 46765) according to the protocol described in the reference 62.

Real Time PCR data was analysed essentially as described earlier (63). In short, average Ct for each sample and ΔCt for IP and input samples were calculated by subtracting average Ct value of control primer set from the test primer set. ΔΔCt was calculated by subtracting the ΔCt of input from ΔCt of IP. The degree of occupancy was calculated by the equation "degree of occupancy = $2^{-\Delta\Delta Ct}$. Values obtained for mock precipitation were also calculated in the same way and considered as background. The actual occupancy (fold enrichment) was expressed against the mock precipitation (Occupancy = degree of occupancy of IP/degree of occupancy of Mock).

RNA isolation and expression analysis

Yeast cells were grown in YPD until OD_{600} was 0.7 and total RNA was isolated using acidic hot phenol method. Specific RNAs were visualized by resolving the primer extension products on gel (24). Intron-specific primers, giving rise to 75bp from pre-tRNA and 5'-end-processed 60bp product were used for *SUP4* primer extension. Intensities of four bands representing total transcripts were quantified in FUJI phosphor imager using Image Gauge software. Primer extension product for a pol II transcribed gene U4 (116 bp) served as internal control.

Co-Immunoprecipitation experiments

Briefly, extract equivalent to 8-10 mg of protein from cells containing HA-tagged H2A.Z or myc-tagged N-terminal truncated Spt16 was incubated with 15μl of protein A sepharose beads and specific antibody (IP) or with IgG Sepharose (mock) at 4^{o}C for 3 hours. Cross-linking was performed with dimethyl-pimelidate (Sigma) to reduce the background. Resin was washed with 25 volumes of ice cold IP150 buffer (26). Bound proteins were eluted by boiling in SDS sample buffer and analyzed by immunoblotting with antibodies against Spt16 or Pob3 or H2A.Z. For in vitro pull-down experiments, Htz1 gene was amplified from yeast genomic DNA and cloned in pET28b for overexpression in *E. coli*. Recombinant 6XHis-tagged H2A.Z was purified using Ni-NTA agarose (Qiagen) as per the manufacturer's protocol. 6XHis-tagged H2A.Z was immobilized on Ni-NTA agarose followed by incubation with purified Spt16 (1:1.5 molar ratio) at 4^{o}C on a rotator for 3 hours. A mock was included where pure Spt16 was incubated with Ni-NTA agarose. Reverse pull-down was similarly carried out with pure Spt16/Pob3 heterodimer, purified from TAP-tagged Spt16 containing yeast cells and immobilized on Calmodulin-Sepharose (Amersham). A mock for this experiment had pure H2A.Z incubated with Calmodulin Sepharose. Beads were washed and bound proteins were analyzed by immunoblotting with anti-Spt16 or anti-H2A.Z antibodies, as above.

Table S1. List of the primers used in this study

Primer name	Sequence 5'-------3'	Used where
FISW2UP	TGGTCATGATGTCGCTATTTCT	Figure 1C, Upstream forward
RISW2UP	TCAAATAATTATCCGGTCCTTCCAA	Figure 1C, Upstream reverse
FISW2DOWN	CCCGGGAGATTTTTTTGTTT	Figure 1C, Downstream forward, Figure 3F
RISW2DOWN	AAAAGAGGCTACAAGAGTTCGTTAAT	Figure 1C, Downstream reverse
ABOXF	ACTCTTTCTTCAACAATTAAATACTCTC	Figure 1C, Gene, forward
RSUP4GENE	CCCGGGGGCGAGTCGAACGCCCGA	Figure 1C, Gene, reverse
TelVIR F	GCGTAACAAAGCCATAATGCCTCC	ChIP, *TELVIR* forward
TelVIR R	CTCGTTAGGATCACGTTCGAATCC	ChIP, *TELVIR* reverse
CMD1UpstreamF	GGGTACTCTGACTGGATAGAAATCT	ChIP, *CMD1* forward
CMD1UpstreamR	CTTCGGTAAGATTGGAGGACATTG	ChIP, *CMD1* reverse
U4 ChIPF	CACTTCCTTCCTTAATACTCCATCC	ChIP, U4 forward
U4 ChIPR	GCAAAAACACAATCTCGGACGAATC	ChIP, U4 reverse
CMD1CodingF	GGCCACTGTGATGAGGTCATTG	Figure S4, *CMD1* RNA
CMD1CodingR	GAGTTGACGAGACATCAGAGCC	Figure S4, *CMD1* RNA
U4 298	CACGGGAAATACGCATATCAGTGA	Figure S4, U4 RNA
U4 299	CCGAATTGACCATGAGGAGACG	Figure S4, U4 RNA
SNR14	GCGAACACCGAATTGACCATG	Primer Extensions, U4 RNA
SUP4 IN	ATCTCAAGATTTCGTAGTGA	Primer Extensions, *SUP4* RNA
FSUP4GENE	CTCTCGGTAGCCAAGTTGGTTTAAGGC	Figure 2D

Table S2. List of yeast strains used in this study

Strain	Phenotype	Genotype	Source
USY6	H3-myc	*MAT***a**, *ura3–52*, *lys2–801*, *ade2–101*, *trp1Δ63*, *his3Δ200*, *leu2Δ1*, *hht1-hhf1Δ*, *hht2-hhf2Δ*, pNOY439 (*CEN6 ARS4 TRP1* MYC-*HHT2-HHF2*)	(64)
YTT196	Isw2Δ	*MAT*a *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 isw2*::*LEU2*	(65)
YM1844	Htz1-HA	*MATa his3Δ,leu2Δ,ura3Δ,lys2Δ,htz1Δ::HA3-HTZ1*	(66)
YM1730	Htz1Δ	*MATα his3Δ0 leu2Δ0 ura3Δ0 lys2Δ0 htz1::Kan*	(66)
SWR1-TAP		*MATa, his3-1, leu2-0, met15-0, ura3-0, SWR1-TAP:HIS*	(67)
MW3993	Rsc4-Δ4	*MATa ura3-52 his3-Δ200 ade2-101 trp1-Δ63 lys2-801 leu2-Δ1 rsc4-Δ4::HIS3*	(31)
MW4019	Rsc4-Δ4, Sth1-Myc	*MATa ura3-52 his3-Δ200 ade2-101 trp1-Δ63 lys2-801 leu2-Δ1 rsc4-Δ4::HIS3, STH1-13Myc:KanMX6*	(31)
FY406	FLAG-tagged H2B	*MAT a hta1-htb1Δ::LEU2, hta2-htb2Δ::TRP1, leu2-Δ1, ura3-52, trp1-Δ63, his3-Δ200/ pJH23 (FB1251) HIS3 CEN ARS HTA1, FLAG-HTB2*	(68)
YBL467	H2A-FLAG	*MATa, ura3-1, lys2Δ::hisG, trp1-1, his3-11, -15, leu2-3, -112, can1-100, Hta1-Flag:LoxP/Hta2-2FLAG:Kan*	(27)
YBL325	Htz1-FLAG	*MATa, ura3-1, lys2Δ::hisG, trp1-1, his3-11, -15, leu2-3, -112, can1-100,Htz1-3xFlagP:LoxP*	(27)
YBL556	Htz1-TAP	*MATa, his3-1, leu2-0, met15-0, ura3-0, Htz1-TAP:HIS*	(27)
YBL557	Htz1-TAP, Swr1Δ	*MATa, his3-1, leu2-0, met15-0, ura3-0, swr1Δ::KanMX6, Htz1-TAP:HIS*	(27)
SJY25	Spt16-myc	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1::kanMX4 SPT16-myc*	(38)
YJW122	Spt16 mutant	*MATa, his3Δ200, leu2Δ r1, lys2-128Δ, ura3-52, trp1Δ63, Spt16Δ922*	(60)
DY7379	Pob3 mutant	*MATα ade2 can1 his3 leu2 lys2 met15 trp1 ura3 pob3-L78R*	(6)
DKY625	Nhp6ΔΔ	*MATα ura3-52trp1-289 his3-Δ1 leu2-3,112 Nhp6AΔ2::URA3 Nhp6BΔ2::HIS3*	(55)
YPB31	Htz1-HA in Chz1Δ	*MATa his3Δ,leu2Δ,ura3Δ,lys2Δ,Chz1Δ,htz1Δ::HA3-HTZ1*	This study
YPB41	Htz1-HA in Spt16Δ922	*MATa, his3Δ200, leu2Δ r1, lys2-128Δ, ura3-52, trp1Δ63, Spt16Δ922,HTZ1-6HA:KANMX4*	This study
YPB17	Nhp6A-myc	*MATa,leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15,Nhp6A-9myc:KANMX4*	This study

Transformation of yeast was carried out as described (69). Chz1 was deleted in Htz1-HA containing strain YM1844 to create the strain YPB31. C-terminal of Htz1 was tagged with HA (70) and transformed into YJW122 strain carrying an N-terminal deletion of the amino acids 3-306 from Spt16 protein (60) to create the strain YPB41. C-terminal of Nhp6A was tagged with 9X myc to create YPB17.

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