Figures and figure supplements

Histone H2B ubiquitylation represses gametogenesis by opposing RSC-dependent chromatin remodeling at the ste11 master regulator locus

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Figure 1. The abolition of histone H2B ubiquitylation suppresses the requirement of Isk1. (A) Spot dilution assay of indicated strains grown 2 days at 32°C on rich medium in the presence or absence of LatA (0.5 μM). The arrow indicates the suppression of Isk1 growth defect by the htb1 KR mutant. Figure 1 continued on next page.
Figure 1 continued

(B) Relative quantification of the cdc14 mRNA determined by quantitative RT-Q-PCR in the indicated strains. a.u.: arbitrary units. Each column represents the averaged value ± SEM (n = 3).

(C) Relative quantification of the ste11 mRNA determined by quantitative RT-Q-PCR in the indicated strains during vegetative growth (T0) and nitrogen starvation at the indicated time points (hours). a.u.: arbitrary units. Each column represents the averaged value ± SEM (n = 3).

(D) The indicated strains were plated for 48 hr on mating medium (malt extract) before iodine staining to reveal sterility.

(E) Left panel. The wt and hrb1 K119R strains were starved for nitrogen at the indicated time points (hours). The occupancy of ubiquitylated H2B at the indicated locations (A, B) was determined by ChIP using the anti-H2Bubi normalized against unmodified H2B. Right panel. The wt and hrb1 K119R strains were starved for nitrogen at the indicated time points (hours). The occupancy of ubiquitylated H2B at the indicated locations (A, B) was determined by ChIP using the anti-H3K4me3 normalized against unmodified H3. The same chromatin sample was used for the left and right panel. Each column represents the averaged value ± SEM (n = 3).

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The overexpression of cdc14 rescues the LatA sensitivity of a lsk1 deleted strain. (A) Spot dilution assay of a wild type and cdc14 ts (cdc14-118, a kind gift of V. Simanis) strains grown 2 days at 25°C on rich medium in the presence or absence of LatA (0.5 μM). Note that Figure 1—figure supplement 1 continued on next page.
25°C is the permissive temperature of the cdc14-118 allele. (B) Spot dilution assay of an lsk1 deleted strain transformed with the empty pREP vector or the same vector expressing the wild type cdc14 gene (a gift of V. Simanis) from the thiamine repressible nmt1 promoter. The strains were grown 2 days on minimal media at 32°C in the presence or absence of LatA (0.5 μM). (C) Spot dilution assay of indicated strains grown 2 days at 32°C on rich medium in the presence or absence of LatA (0.5 μM). (D) Relative quantification of the ste11 mRNA determined by quantitative RT-Q-PCR in the indicated strains during vegetative growth (T0) and nitrogen starvation at the indicated time points (hours). a.u.: arbitrary units. Each column represents the averaged value ± SEM (n = 2)

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A

H. sapiens
MPEPAKAPAPKKSSKAVTKAQKDSKRKSRSRYEYVYVYKVLKQVHPDTGISKAM 60
S. pombe
MSAAKE-KPAKAPAGAPRDMKSDKGRKRNKRETYSYIYKVLKQVHPDTGISAQAM 59

*..*  **.* : ** : . *..** :***:*  :*********::*

H. sapiens
GIMNSFVNDIPERIAGEASRLAHYNKRSTTITSREIQTAVRLLPGLAKHAVSEGTRAUT 120
S. pombe
RILNSFVNDIPERIATEASKLAAYNKSTISSLREIQTAVRLILPGLAKHAVTEGKSVT 119

*........*  ***  ***  *:*  **:* **:********:****:

S. pombe
KVSQ  126

B

B

C

1  ATGTCCTGCTGCTGAAGAGAAGGAGGGGCTCCAAGGGCTCCGCGGAGAAGAGCTAGGGAT
  1  MSAAAEKKKAPASKAKAPAGAPR0DKSDKGRKRNKRETYSYI

51  ACCATGAAGAGTGCCTGATAAGAAAGCCGCTGTAAGAAACAGAAAGGAAACTTAATTTATCTCCCTAT
  21  TMKSAADKKKRGRKRNKIRETYS

121  ATTTAAAGTTGTTAGGAAAGCTACTCCCTGTAATCTGATTTCCACAAGCGCCATCTGGCT
  41  IYKVLKQVHPDTGISNQAMR

181  ATCTTGAACTCTTTGCACACGATATTTTGTGACTGCCACTGAGGCTCTCAACAGCTTT
  61  IILNSFVNDIPERIATEASKL

241  GCTGCTAACAAACAAGGCTTCCACTATTTTCTTCCCGTGAAATCCAGACTGCTGTTGTTTG
  81  AAAYNKKSTISSREIQTAVRL

301  ATCTTCCCGTGTAAGAAAAAGCTACTACCCGCTGAGGCTTACCAGAACATCCCCATCAAG
  101  IILPGELAKHAVTeGTXKSVT

361  TATCTCCTCTCTCCTCAGTAAA

121  YSSSAQ *

ACT

T

GCC

A
Figure 1—figure supplement 2. Characterization of the histone H2B mutants generated. (A) Sequence alignment of the histone H2B gene from Human (H. sapiens) and fission yeast (S. pombe). The ubiquitylated site (K120 in Human, K119 in fission yeast) is circled in red. Note that the numbering takes into account the removal of the first methionine. (B) Western blot analysis (anti-H2B and anti-H2Bubi) of total protein extracts from the indicated strains. KR: htb1 K119R, SA-ST: htb1 S116A S121T, SA-ST-KR: htb1 S116A K119R S121T (see A) for sequence details. The * designs a non-specific band. Quantification of signal intensity was performed from scanned gels using Image J and the numbers represent the signal obtained with the anti-H2Bubi normalized on the signal obtained with the anti-H2B with the wild type set as 1. (C) DNA and protein sequences of the fission yeast htb1 gene encoding histone H2B. The epitope used to generate the antibody is framed. The S116A and S121T mutation designed to ‘humanize’ the fission yeast gene are indicated in red. Note that the numbering takes into account the removal of the first methionine.

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Figure 2. The RSC complex is required for the induction of ste11. (A) The occupancy of histone H3 at the ste11 promoter was measured by ChIP using the indicated amplicon in the indicated strains. Each column represents the averaged value ± SEM (n = 3). (B) Relative quantification of the ste11 mRNA
determined by quantitative RT-Q-PCR in the indicated strains during vegetative growth (T0) and nitrogen starvation at the indicated time points (hours). a.u.: arbitrary units. Each column represents the averaged value ± SEM (n = 3). (C) Western blot analysis (anti-TAP and anti-tubulin) of total protein extracts from the indicated strains grown 2 hr in the presence or absence of anhydrotetracycline (ahTet 2.5 µg/ml). (D) Spot dilution assay of indicated strains grown 2 days at 32˚C on rich medium in the presence or absence of ahTet (2.5 µg/ml). (E) Relative quantification of the snf21 mRNA determined by quantitative RT-Q-PCR in the indicated strain grown in the presence of ahTet for three hours. Samples were taken at the indicated time (hours). a.u.: arbitrary units. Each column represents the averaged value ± SEM (n = 3). (F) Same as E, except that the ste11 mRNA was quantified. Each column represents the averaged value ± SEM (n = 3). (G) The occupancy of histone H3 at the ste11 promoter was measured by ChIP using the indicated amplicon in the tet0-snf21-TAP strain grown in the presence of ahTet for two hours. Each column represents the averaged value ± SEM (n = 3). DOI: 10.7554/eLife.13500.005
Figure 2—figure supplement 1. Subunits composition of the fission yeast SWI/SNF and RSC complexes - Schematic of the rTetR switch off system used. (A) The subunit composition of the SWI/SNF and RSC chromatin remodeling complexes is indicated with the shared subunits. The red colour

Figure 2—figure supplement 1 continued on next page
Figure 2—figure supplement 1 continued

refers to essential subunits (Monahan et al., 2008). (B) The indicated homothallic strains were plated for 48 hr on mating medium (malt extract) before iodine staining to reveal sterility. (C) Relative quantification of the cdc14 mRNA determined by quantitative RT-Q-PCR in the indicated strains during vegetative growth. a.u.: arbitrary units. Each column represents the averaged value ± SEM (n = 2). (D) The occupancy of Snf22-TAP at the ste11 and cdc14 promoters was measured by ChIP using the indicated amplicons in the wt and Snf22-TAP strains. Each column represents the averaged value ± SEM (n = 3). (E) A TAP tag encoding sequence was integrated at the snf21 locus. The tetO operator was then integrated as described (Zilio et al., 2012), which allows rTetR repression in the presence of ahTet.

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Figure 3. The RSC complex regulates nucleosomes occupancy at the ste11 promoter. (A) The occupancy of histone H3 at the ste11 locus was measured by ChIP using the indicated amplicons (A–D) in the indicated strains. Each column represents the averaged value ± SEM (n = 3). (B) Nucleosome scanning analysis of the indicated strains. Nucleosomal DNA enrichment at the indicated positions of the ste11 locus was determined by ChIP experiment on MNase-digested chromatin. Data are presented as the average of three independent experiments along with the SEM. Inferred nucleosome locations are indicated. The bar indicates the position of amplicon A used in ChIP experiments. (C) The occupancy of Rsc1-TAP at the ste11 locus was measured by ChIP using the indicated amplicons (A–B) in the wt and htt2 K14R strains. Each column represents the averaged value ± SEM (n = 4). To determine whether the decreased Rsc1-TAP enrichment was statistically significant, the difference in the means of enrichment between the ChIP peak in the wild type and the tested strains was estimated using t-test, assuming unequal variances between samples (Welch’s t-test). p-value < 0.05 indicated by *, n.s.: non significant. (D) The occupancy of Rsc1-TAP at the ste11 locus was measured by ChIP using the indicated amplicons (A–B) in the wt and lsk1 deleted strains. Each column represents the averaged value ± SEM (n = 4). To determine whether the decreased Rsc1-TAP enrichment was statistically significant, the difference in the means of enrichment between the ChIP peak in the wild type and the tested strains was estimated using t-test, assuming unequal variances between samples (Welch’s t-test). p-value < 0.05 indicated by *, n.s.: non significant.

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Figure 3—figure supplement 1. Schematic of amplicons used in the nucleosome scanning experiments. The black bars represent 17 overlapping amplicons covering a 1179 bp region encompassing the NDR and 215 bp of theste11 transcribed region. The +1 site and its genomic coordinate are indicated. The red bar represents the amplicon used in ChIP experiments (referred as A).
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Figure 3—figure supplement 2. The NuA3 and NuA4 HATs and histone H4 acetylation on K16 are not required for ste11 induction. (A) Relative quantification of the ste11 mRNA determined by quantitative RT-Q-PCR in the indicated strains grown during vegetative growth (T0, 25°C) and nitrogen starvation at the indicated time points (hours) at 36°C. Note that the strains were shifted to 36°C one hour before nitrogen starvation. Samples were taken at the indicated time (hours). a.u.: arbitrary units. Each column represents the averaged value ± SEM (n = 2).

(B) Relative quantification of the ste11 mRNA determined by quantitative RT-Q-PCR in the indicated strains grown during vegetative growth (T0) and nitrogen starvation at the indicated time points (hours). a.u.: arbitrary units. Each column represents the averaged value ± SEM (n = 2).

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Figure 4. H2B-ubi and HDAC oppose the recruitment of the RSC complex at the ste11 promoter. (A) The occupancy of ubiquitylated H2B at the indicated locations (A, B) was determined by ChIP in the indicated strains using the anti-H2Bubi normalized against unmodified H2B. Each column
Figure 4 continued

represents the averaged value ± SEM (n = 3). (B) The occupancy of Arp9-TAP at the ste11 locus was measured by ChIP using the indicated amplicons (A-B) in the wt and htb1 K119R strains. Each column represents the averaged value ± SEM (n = 3). (C) The occupancy of Arp9-TAP at the ste11 locus was measured by ChIP using the indicated amplicons (A–B) in the wt and hos2A strains. Each column represents the averaged value ± SEM (n = 3). (D) The occupancy of Arp9-TAP at the ste11 locus was measured by ChIP using the indicated amplicons (A–B) in the wt and lsk1A strains. Each column represents the averaged value ± SEM (n = 3). (E) The occupancy of acetylated H3K14 at the ste11 promoter was determined by ChIP in the indicated strains using the anti-H3 K14ac normalized against unmodified H3. Each column represents the averaged value ± SEM (n = 3). DOI: 10.7554/eLife.13500.010
A

![Graph A](image1)

B

![Graph B](image2)

C

![Graph C](image3)

D

![Graph D](image4)

E

![Graph E](image5)
Figure 4—figure supplement 1. Genome-wide analysis of the absence of Rsc1 on NDR and connection between RSC and CTD S2P. (A) Left panel: Meta-gene analysis of the nucleosome occupancy signal for all protein coding genes near the TSS for the wt and the rsc1 mutant. Right panel: distribution of the variation in NDR length at TSS between wt and the rsc1 mutant. The 10% genes showing the strongest increase or strongest decrease in NDR size are indicated. (B) Left panel: Meta-gene analysis of the nucleosome occupancy signal for the 10% protein coding genes showing the strongest decrease in NDR size at the TSS in the rsc1 mutant compared to wild type. The distance between the TSS and the average -1 nucleosome midpoint is indicated in blue for the wt and red for the rsc1 mutant. (C) Nucleosomes occupancy nearby the transcription start site of ste11 (blue: wt, red: rsc1). (D) Left panel: Meta-gene analysis of the nucleosome occupancy signal of the previously established list of genes whose promoter nucleosome-depleted region (NDR) shrinks the most in the S2A mutant (Materne et al., 2015) when rsc1 is deleted. Right panel: Venn diagram showing the overlap between the number of fission yeast coding genes with the strongest 10% shrinkage in the rsc1 and CTD S2A mutants. Enrichment was computed as Fisher’s exact one-sided test p-value.

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The following source data is available for figure 4:

Figure supplement 1—Source data 1. List of the 10% genes showing the strongest promoter NDR size decrease in the rsc1 mutant (see Figure 4—figure supplement 1A, right panel) with their genomic coordinates, NDR size in wild type and the rsc1 mutant.

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Figure 4—figure supplement 2. Schematic of the role of H2B-ub1 and RSC in the control of \textit{ste11} expression. (A) See text for details. The grey line marked with an asterisk refers to the reported repressing role of H2Bubi on Ctk1 recruitment (Wyce et al., 2007). (B) The occupancy of Lsk1-TAP at the \textit{ste11} promoter was measured by ChIP in the indicated strains. Each column represents the averaged value ± SEM (n = 3). DOI: 10.7554/eLife.13500.013