Glutamine methylation in histone H2A is an RNA–polymerase–I–dedicated modification

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Nucleosomes are decorated with numerous post-translational modifications capable of influencing many DNA processes. Here we describe a new class of histone modification, methylation of glutamine, occurring on yeast histone H2A at position 105 (Q105) and human H2A at Q104. We identify Nop1 as the methyltransferase in yeast and demonstrate that fibrillarin is the orthologue enzyme in human cells. Glutamine methylation of H2A is restricted to the nucleolus. Global analysis in yeast, using an H2AQ105me-specific antibody, shows that this modification is exclusively enriched over the 35S ribosomal DNA transcriptional unit. We show that the Q105 residue is part of the binding site for the histone chaperone FACT (facilitator of chromatin transcription) complex. Methylation of Q105 or its substitution to alanine disrupts binding to FACT in vitro. A yeast strain mutated at Q105 shows reduced histone incorporation and increased transcription at the ribosomal DNA locus. These features are phenocopied by mutations in FACT complex components. Together these data identify glutamine methylation of H2A as the first histone epigenetic mark dedicated to a specific RNA polymerase and define its function as a regulator of FACT interaction with nucleosomes.

Glutamine methylation occurs on translation termination factors and ribosomal proteins. We investigated whether such a modification exists on histones by interrogating mass spectrometric data sets. We identified a single glutamine, human Q104 (yeast: Q105) in H2A as a site of methylation (Fig. 1a and Extended Data Fig. 1). The residue is located on the surface of the octamer (Extended Data Fig. 2) and is highly conserved in canonical H2A from yeast to human. However, in H2A.Z it is exchanged for a glycine or serine (Fig. 1b). We raised a modification-specific antibody (Extended Data Fig. 3) that detects this modification in yeast and mammalian cells (Fig. 1c).

To identify the methyltransferase responsible, we performed a candidate approach and screened 72 predicted yeast non-essential predicted methyltransferases by analysing knockout lysates by western blotting with the modification-specific antibody. However, we did not detect loss of signal in any lysate (not shown). We then used an unbiased biochemical approach and fractionated yeast cells as described in Fig. 2a. Fractions were assayed on a 20-residue peptide spanning Q105, or the respective QA mutant, coupled to beads in the presence of tritiated S-adenosyl-methionine (SAM). Methyltransferase activity was assessed by scintillation counting (Fig. 2b) and the fraction containing activity towards H2AQ105 was subjected to mass spectrometry (Supplementary Table 4). All 178 non-essential proteins identified by mass spectrometry were tested by knockout analysis and enzymatic activity was assessed by scintillation counting (Fig. 2b) and the fraction containing activity towards H2AQ105 was subjected to mass spectrometry (Supplementary Table 4). All 178 non-essential proteins identified by mass spectrometry were tested by knockout analysis and enzymatic activity was assessed by scintillation counting (Fig. 2b) and the fraction containing activity towards H2AQ105 was subjected to mass spectrometry (Supplementary Table 4).

Figure 1 | Identification and localization of methylated H2A glutamine 105. a, Tandem mass spectrum of the Q104me modified peptide. b, Alignment of the region encompassing Q105 of H2A and its variant H2A.Z. Highlighted in red is Q105 in H2A and the corresponding change to glycine or serine in H2A.Z. c, Analysis of yeast and mammalian cell extracts for the presence of Q105 methylation using a modification-specific antibody.

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Figure 2 | Identification of Nop1/fibrillarin as the methyltransferase of Q105.

Data Fig. 4b). To test the enzymatic activity of Nop1 in vivo we made use of two independently isolated thermosensitive mutants carrying the same amino-acid changes, which are located in the SAM binding site of Nop1 (ref. 5). Yeast harbouring these thermosensitive (ts) alleles showed a 50% reduced Q105 methylation signal upon shift to restrictive temperature at a time at which cells are still proliferating (Fig. 2f and Extended Data Fig. 5e). At this time viability was only marginally affected, based on MTT proliferation assays (Extended Data Fig. 6). Remarkably, when the H2AQ105me antibody was stained using the Q105me-specific and anti-fibrillarin antibodies and counterstained with 4’,6-diamidino-2-phenylindole (DAPI) as a nuclear marker.

However, siRNA-induced knockdown of fibrillarin completely abrogated detection of Q104me in the nucleolus. We observed no morphological changes of the nucleus and nucleolus that have been reported to occur upon prolonged fibrillarin knockdown14, indicating that—in agreement with the MTT assay—the viability of the cells was not affected at the time of analysis.

The main function of the nucleolus is ribosomal DNA (rDNA) transcription and ribosome biogenesis15. To analyse the distribution of H2A glutamine methylation in chromatin, we performed chromatin immunoprecipitations coupled to deep DNA sequencing (ChIP-seq). The rDNA locus consists of roughly 100–200 repeats in yeast and 200–400 copies in human cells13, of which about half are active and almost devoid of nucleosomal structure and the other half are inactive and densely packed with nucleosomes13,14. In yeast up to about 80% of rDNA repeats can be deleted, in which case all the remaining repeats, approximately 20 copies, are active15. This strain still retains H2AQ105me (Extended Data Fig. 6). Remarkably, when the H2AQ105me antibody
In ChIP-seq analysis, the only site of enrichment in the entire yeast genome is over the 35S rDNA transcription units (Fig. 3a, c). In addition, Nop1, the enzyme that mediates H2AQ105 methylation, co-localizes with H2AQ105 methylation at the 35S rDNA locus (Fig. 3d). Together, these results indicate that both in human and in yeast, methylation of H2A represents a modification restricted to the nucleolus. Given the enrichment of glutamine methylation on the transcribed region of the rDNA cluster, we asked whether RNA polymerase I transcription was required for deposition of Q105 methylation. We used 35S rRNA as a reporter to scan the rDNA region by ChIP–quantitative (q)PCR. The ChIP–qPCR validation of the ChIP-seq shown in a, d, Nop1 profile over the rDNA locus. The ChIP–qPCR profile was internally normalized to signal of primer pair ‘A’. ChIP–qPCR data show the mean ± s.e.m. of three independent biological experiments.

Histone methylation can act as a platform to recruit and regulate other chromatin-related factors such as chromatin remodelling complexes. The region spanning Q105 in H2A has previously been described as a potential binding site for FACT, a protein complex consisting of Spt16/Pob3 and Nhp6a in yeast, which is required for efficient passage of RNA and DNA polymerases through chromatin by remodelling nucleosomes. Residues in the region of H2A spanning Q105 show genetic interactions with FACT ts mutants, as does a Q105A mutant using a transcription-based reporter system (Extended Data Fig. 8). To probe the possibility that FACT physically interacts with H2A through the region spanning Q105, we took an unbiased approach (phage display) to identify regions in H2A/H2B interacting with Spt16 and Pob3. A randomized 12-residue peptide phage library was used to enrich for sequences binding to Spt16/Pob3. In line with the published genetic findings, the interaction screen identified a consensus sequence spanning H2AQ105 as the binding site for FACT (Extended Data Fig. 9a).

We next asked whether methylation of H2AQ105 could influence the binding to FACT. Figure 4a shows that binding of recombinant Spt16/Pob3 to a peptide spanning H2AQ105 is significantly decreased when Q105 is methylated or mutated to alanine. Pull-downs from HeLa nuclear extracts using the same peptides demonstrate that the endogenous human FACT complex is responsive to glutamine methylation on H2A (Extended Data Fig. 9b), suggesting that the disruption...
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the Q105A and FACT

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in terms of effecting FACT binding (Fig. 4a). To study a potential

fluence of Q105 methylation on rDNA transcription and its interplay

with FACT, we turned to a well-established reporter-based system that

allows sensitive monitoring of the transcriptional state of the rDNA

locus, in which weak, but constitutively expressed, URA3 cassettes were

integrated into the rDNA locus (S3 and S6; Fig. 4b)19. Figure 4b shows a

significant drop in 5-FOA-positive colonies in the Q105A mutant, indic-

ative of a higher transcription rate of the UR3A. We then introduced the

spt16-11 allele—which leads to a 30–40% decrease in FACT protein

levels17—into the same reporter strains. Figure 4d shows that at semi-

permisiveness at the rDNA locus. To monitor RNA Pol I transcription

rupts the binding of FACT to chromatin lead to transcriptional per-

missiveness (open chromatin) in accordance with recently published

findings20. These results show that reduced FACT activity indeedphe-

nocopies the Q105A mutation. Thus, a mutation that disrupts

the function of the chromatin remodeler FACT or a mutation that dis-

rupts the binding of FACT to chromatin lead to transcriptional per-

missiveness at the rDNA locus. To monitor RNA Pol I transcription

rates directly, we performed run-on experiments in wild-type, FACT

ts

and Q105A strains. Figure 4d shows that rDNA transcription was

increased using two different primer pairs, confirming a more permis-

siveness at the rDNA locus, when FACT or Q105A are mutated.

One possible explanation for the increased rDNA transcription in

the Q105A and FACT ts strains, is loss of nucleosomes over a transcribed

region. FACT ts mutants have already been described as possessing such

a phenotype21. To investigate a possible histone deposition defect, we

generated strains in which we placed myc-tagged versions of either wild-

type H2A or the Q105A mutant under control of the GAL1-promoter in a

wild-type or spt16-11 yeast background. Induction of the myc-tagged

histones was identical as judged by total steady-state levels on western

blots (Extended Data Fig. 10a). The wild-type H2A was very efficiently

deposited into chromatin as monitored by ChIP (Fig. 4e). However, the

spt16-11 and the Q105A mutant had a profound defect in H2A incor-

poration into chromatin (Fig. 4e). These findings suggest that methyla-

tion of H2AQ105, as phenocopied by the H2AQ105A mutation and a FACT

ts mutant, results in the transcriptional stimulation of the UR3A repor-

ters because of reduced nucleosomal occupancy in the rDNA repeat.

Finally, we set out to test directly whether an increase in Q105 methyla-

tion on the rDNA locus would decrease FACT occupancy. Indeed, overexpression of Nop1 leads to increased Q105me and is accompanied by a decrease of FACT occupancy, in line with the hypothesis that Q105 methylation is regulating FACT availability on chromatin (Fig. 4f).

The findings presented here identify a new histone modification pathway operatively exclusively in the nucleolus. It involves methylation of H2AQ105 by Nop1 in yeast, and methylation of H2AQ104 by fibrillar

in human cells, resulting in the weakening of interactions between H2A and FACT. The FACT complex interacts with all three RNA polymerases22,23 and facilitates transcription in two steps by (1) bind-

ing and disrupting nucleosomes in the path of the polymerase22,24,25

and (2) by augmenting the re-deposition of nucleosomes in the wake

of transcriptional polymerase22,24,25. Glutamine methylation of H2A may

affect either of these functions by disrupting binding to FACT. The

observation that an H2AQ105A mutant is incorporated to a lower extent

would favour a model in which re-deposition is decreased. Such a model is also in agreement with earlier observations that rDNA has a low nucle-

osome occupancy13, in contrast to most other regions of the genome.

Indeed, recent reports suggest that H2A in particular seems to be depleted from this region27 and that FACT might play a role in this pathway20. The net result of a glutamine-modified chromatin state is that RNA Pol I transits less impeded by nucleosomes. It is worth noting, how-

ever, that glutamine methylation of H2A is present in the rDNA locus, even though deposition is affected. The residual loading of modified H2A might be due to the presence of other histone chaperones such as

Nap1 that are insensitive to glutamine methylation on H2A (Extended Data Fig. 10b).

RNA Pol I associates with the Nop1 enzyme and may carry it along during transcription elongation. It is currently unclear how glutamine methylation is initiated or reversed, but it might be linked to the re-

activation of the rDNA locus that has been described to occur after DNA replication in an RNA Pol-I-dependent manner27. Another pos-

sibility is that the enzyme itself is the key node of regulation. Nop1/ fibrillarin is highly modified28,29, so a signalling pathway leading to the glutamine methyltransferase could be the triggering event.

Glutamine methylation of H2A represents the first histone modi-

fication that is dedicated to only one of the three RNA polymerases. The selectivity for Pol I and its compartmentalization within the nucle-

loseus might be necessary to generate a chromatin state capable of deal-

ing with the high demands for transcription of ribosomal components. Indeed, glutamine methylation as a whole seems to be a modification that is dedicated to ribosomal biosynthesis: Nop1 has the ability also to methylate rRNA and affect RNA processing3; the only other known glutamine methyltransferases in yeast (Mtx1 and Mtx2) modify translational release factors on a conserved glutamine3. Thus, glutamine met-

thylation may have evolved to be a modification dedicated to a specific cellular process. Finally, our finding that a protein can catalyse the methylation of proteins and RNA opens the possibility that many other enzymes may have such dual specificity.

METHODS SUMMARY

The antibody against H2AQ105me was raised using the speedy 28-day programme of Eurogentec using modified peptides coupled to KLH. Yeast genetics, molecular biology, cell culture and biochemistry were performed using standard methods. ChIP was essentially as described earlier20. ChIP-qPCR was analysed on an Illumina MiSeq. Mapped ChIP-seq reads were normalized by dividing Q105me counts by the H2A counts. Mass spectrometry was performed upon in-gel digest on a LTQ-Orbitrap XL (Thermo Fisher Scientific) and analysed using the MaxQuant software package. Detailed information about the reagents and methodology used is available in Methods.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Kouzarides, T. Chromatin modifications and their function. Cell 128, 693–705 (2007).

2. Formosa, T. The role of FACT in making and breaking nucleosomes. Biochim. Biophys. Acta 1813, 247–255 (2012).

3. Polevoda, B. & Sherman, F. Methylation of proteins involved in translation. Mol. Microbiol. 65, 590–606 (2007).

4. Petrossian, T. C. & Clarke, S. G. Multiple motif scanning to identify methyltransferases from the yeast proteome. Mol. Cell. Proteomics 8, 1516–1526 (2009).

5. Tollery, D., Lehtonen, H., Jansen, R., Kem, H. & Hurt, E. C. Temperature-sensitive mutations demonstrate roles for yeast fibrillarin in pre-rRNA processing, pre-rRNA methylation, and ribosome assembly. Cell 72, 443–457 (1993).

6. Gautier, T., Bergès, T., Tollery, D. & Hurt, E. Nucleolar KKE/D repeat proteins Nop55p and Nop58p interact with Nop1p and are required for ribosome biogenesis. Mol. Cell. Biol. 17, 7088–7098 (1997).

7. Fath, S. et al. Association of yeast RNA polymerase I with a nucleolar substructure active in rRNA synthesis and processing. J. Cell Biol. 149, 575–590 (2000).

8. Krogan, N. J. et al. Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. Nature 440, 637–643 (2006).

9. Lambert, J.-P., Mitchell, L., Rudner, A., Baetz, K. & Figyes, D. A novel proteomics approach for the discovery of chromatin-associated protein networks. Mol. Cell. Proteomics 8, 870–882 (2009).

10. Jansen, R. P. et al. Evolutionary conservation of the human nucleolar protein fibrillarin and its functional expression in yeast. J. Cell Biol. 113, 715–729 (1991).

11. Amin, M. A. et al. Fibrillarin, a nucleolar protein, is required for normal nuclear morphology and cellular growth in HeLa cells. Biochem. Biophys. Res. Commun. 360, 320–326 (2007).

12. Ratak, I., Shaw, P. J. & Cmarko, D. Structure and function of the nucleolus in the spotlight. Curr. Opin. Cell Biol. 18, 325–334 (2006).

13. Alberti, B., Perez-Fernandez, J., Lager-Silvestre, I. & Gadai, O. Regulation of ribosomal RNA production by RNA polymerase I: does elongation come first? Genet. Res. Int. 2012, 276948 (2012).
14. Grummt, I. & Längst, G. Epigenetic control of RNA polymerase I transcription in mammalian cells. Biochim. Biophys. Acta 1829, 393–404 (2013).
15. Ide, S., Miyazaki, T., Maki, H. & Kobayashi, T. Abundance of ribosomal RNA gene copies maintains genome integrity. Science 327, 693–696 (2010).
16. Gorenstein, C., Atkinson, K. D. & Falkes, E. V. Isolation and characterization of an actinomycin D-sensitive mutant of Saccharomyces cerevisiae. J. Bacteriol. 136, 142–147 (1978).
17. VanDemark, A. P. et al. Structural and functional analysis of the Spt16p N-terminal domain reveals overlapping roles of yFACT subunits. J. Biol. Chem. 283, 5058–5068 (2008).
18. McCullough, L. et al. Insight into the mechanism of nucleosome reorganization from histone mutants that suppress defects in the FACT histone chaperone. Genetics 188, 835–846 (2011).
19. Smith, J. S. & Boeke, J. D. An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev. 11, 241–254 (1997).
20. Johnson, J. M. et al. Rpd3 and Spt16-mediated nucleosome assembly and transcriptional regulation on yeast rDNA genes. Mol. Cell. Biol. 33, 2748–2759 (2013).
21. Hainer, S. J., Pruneski, J. A., Mitchell, R. D., Monteverde, R. M. & Martens, J. A. Intergenic transcription causes repression by directing nucleosome assembly. Genes Dev. 25, 29–40 (2011).
22. Orphanides, G., Wu, W. H., Lane, W. S., Hampsey, M. & Reinberg, D. The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature 400, 284–288 (1999).
23. Birch, J. L. et al. FACT facilitates chromatin transcription by RNA polymerases I and III. EMBO J. 28, 854–865 (2009).
24. Belotserkovskaya, R. et al. FACT facilitates transcription-dependent nucleosome alteration. Science 301, 1090–1093 (2003).
25. Winkler, D. D., Muthurajan, U. M., Hieb, A. R. & Luger, K. Histone chaperone FACT coordinates nucleosome interaction through multiple synergistic binding events. J. Biol. Chem. 286, 41883–41892 (2011).
26. Formosa, T. et al. Defects in SPT16 or POB3 (yFACT) in Saccharomyces cerevisiae cause dependence on the Hir/Hpc pathway: polymerase passage may degrade chromatin structure. Genetics 162, 1557–1571 (2002).
27. Wittner, M. et al. Establishment and maintenance of alternative chromatin states at a multicyclic gene locus. Cell 145, 543–554 (2011).
28. Choudhary, C. et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science 325, 834–840 (2009).
29. Wang, B., Malik, R., Nigg, E. A. & Körner, R. Evaluation of the low-specificity protease elastase for large-scale phosphoproteome analysis. Anal. Chem. 80, 9526–9533 (2008).
30. Santos-Rosa, H. et al. Methylation of histone H3 K4 mediates association of the Isw1p ATPase with chromatin. Mol. Cell 12, 1326–1332 (2003).

Supplementary Information is available in the online version of the paper.

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Author Contributions P.T. and H.S.-R. designed experiments, performed research, interpreted data and wrote the manuscript. K.B.S. and M.L.N. performed mass spectrometry. C.J.N. supplied new reagents. T.K. designed experiments, interpreted data and wrote the manuscript.

Author Information Data of the ChIP-seq experiments have been deposited in Array Express under accession number E-MTAB-1447. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to T.K. (t.kouzarides@gurdon.cam.ac.uk).
**METHODS**

**Strains, plasmids and reagents.** Genotypes of strains and yeast plasmids used in this work are listed in Supplementary Table 1. If not stated otherwise, all strains used were derivatives of W303. Integrations and deletions were performed using one-step PCR-based methods. All plasmids used were verified by sequencing. Non-radioactive SAM and recombinant histones were purchased from New England Biolabs and tritiated SAM from Perkin Elmer. Peptides for immunization were from ChemPep and biotinylated peptides were from Cambridge Peptides using Fmoc-Gln(Me)-OH (ChemPep). Ni-NTA and IgG Sepharose were from GE Healthcare, amylene resin from New England Biolabs and streptactin-sepharose from iBA. Streptavidine, protein A and protein G Dynabeads were from Invitrogen. Anti-SSRP1 (D-7) and Anti-myc (9E10 for ChIP), rabbit anti-c-myc (C3956 for western blot), anti-biotin fibrillarin (ab5821 for western blot) and mouse anti-HA (ab1424) were from Abcam. For Fig. 1c) mouse anti-fibrillarin (ab4566 for immunofluorescence), rabbit anti-SSRP1 (D-7) and anti-CDK16/Pob3 (10 nm final) in 500 μl TBS/0.1% NP40, 1 mM DTT for 2 h at 4 °C. Subsequently, beads were washed four times with 1 ml TBS/0.1% NP40 1 mM DTT and eluted with 50 μl SDS-sample buffer.

**Phage display assay.** We used the Ph.D.-12 Phage Display Peptide Library Kit from New England Biolabs to screen for a potential Spt16/Pob3 interaction site in H2A using the manufacturer’s instructions. As a bait we used Hi6-tagged Spt16/StreptI-Pob3. In the first selection round we used Ni-NTA, in the second streptactin and in the third round we used both matrices for affinity purification. DNA of enriched phages was prepared and sequenced using the kit’s primer.

**ChIP.** Chromatin was sonicated to produce fragments of 400–500 base pairs (bp). ChIP was performed essentially as described, except ChIPs for H2AQ105me, in which case five times more chromatin was used as input. Real-time PCR used a StepOnePlus system with Fast SYBR Green (Applied Biosystems). Standard curves for each primer set were calculated from amplification of diluted DNA. After each run, a dissociation curve was performed to ensure that no primer dimers contaminated the quantification and that the product had the expected melting temperature. Each PCR reaction was performed in duplicate and the signal intensity value for each sample was calculated from the average of the two experiments. Primer sequences are shown in Supplementary Table 2. Relative fluorescent intensities for the ChIP experiments were calculated as follows: (Ab signal — IgG signal)/(input signal — IgG signal), where Ab is the antibody of interest, IgG is the negative control antibody and input is the sheared genomic DNA. Each experiment was repeated between two and four times from independent cultures.

**ChIP-seq.** Fifty per cent of a ChIP reaction was used to generate the library. Library production followed the protocol published on http://ethanomics.wordpress.com/ chip-seq-library-construction-using-the-illumina-truseq-adapters/ (2012) with few changes. Purification steps using AMPure XP beads were replaced by the ChIP DNA Clean & Concentrator kit by ZymoResearch. Illumina adaptors were exchanged for Biso Nextflex adaptors 1–3 (1:100 dilution each). Size selection was performed on a SAGE Pippin Prep using 2% gels and selecting for a size between 350 and 600 bp. The quality of the library was assessed using Agilent DNA High Sensitivity ChIPs and Qagen Qubit. Libraries were pooled and sequenced on an Illumina MiSeq using single-ended 50 bp reads.

**Transcriptional run-on.** Nuclear Run-On was performed essentially as described earlier. Briefly, cells were inoculated to a starting absorbance (A600 nm) of approximately 0.03 and grown in YPAD medium to A600 nm ~ 0.1–0.15. Five A600 nm units of culture (approximately 5 x 10^6 cells) were collected by centrifugation at 3,000g for 3 min. Cells were resuspended in 5 ml of ice-cold H2O and then centrifuged 4 for 3 min at 4 °C. The cells were then re-suspended in 950 μl of ice-cold H2O, and 50 μl of 10% sarkosyl was added. The sample was gently mixed and centrifuged 4 for 20 min. The resuspended cells were pelleted by microcentrifugation for 1 min, and the supernatant was carefully removed. Residual supernatant was removed after a second brief microcentrifugation.

The cells were suspended in 79.5 μl of transcription buffer (50 mM Tris hydrochloride (pH 8), 100 mM KCl, 5 mM MgCl2, 1 mM MnCl2, 2 mM DTT) supplemented with 40 U of RNase OUT (Invitrogen). Reactions were started by adding 2.5 μl of RNA biotin labelling mix (Roche) and shifting to 30 °C. After 5 min the reaction was pipetted into a fresh Eppendorf tube with 250 μl glass beads and 1 ml TRizol reagent (Invitrogen) on ice. Cells were lysed and homogenized by bead beating 4 for 1 min with incubation on ice for 3 minutes in between. After the last bead beating step the resuspended cells were pelleted by microcentrifugation for 1 min, and the supernatant was carefully removed. Residual supernatant was removed after a second brief microcentrifugation.

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0.015 M sodium citrate), 15% formamide for 15 min and once in 500 µl of 2× SSC for 5 min. Beads were resuspended in 12 µl RNase free water and RNA was reverse transcribed. Sequences of primers used for qPCR are given in Supplementary Table 2.

Sequence mapping. Called reads from the Illumina MiSeq system were mapped to the latest version of the Saccharomyces cerevisiae genome (sacCer3) using Burrows Wheeler Aligner with parameters -n 10, -k 3, -l 20 (ref. 34). PCR duplicates mapping to identical regions on the genome were removed using SamTools35. Filtered mapped reads were extended to the fragment length of 400 bp and converted to wiggle format using the bedTools suite of utilities36. Raw and mapped reads are given in Supplementary Table 3.

Normalization. Extended reads were counted in bins of 50 bp across the entire genome and were normalized to the total number of mapped reads for each sample to account for differences in read depth. Within each bin, the level of Q105me enrichment above H2A control was determined by first subtracting the IgG read count and subsequently thresholding at a minimum value of one (adjusted value). The normalized Q105me count was generated by dividing the adjusted Q105me values by the adjusted H2A count. Data were transformed to a log2 scale for visualization.

Western blot. Western blots of total yeast extracts were performed by standard procedures. Transfers to nitrocellulose membranes were made on carbonate buffer (21.1 g1 NaHCO3, 18.35 g1 Na2CO3, pH 9.5) at 45 mA for 70 min. The membranes were blocked for 1 h at room temperature in TBS + 0.1% Tween-20 + 5% BSA; primary antibody was incubated at 4 °C overnight and secondary antibody for 1 h at room temperature.

Cell culture. MCF 10A (CRL-10317) cells were obtained from the American Type Culture Collection and were grown in DMEM-H/F12 medium supplemented with 1% penicillin–streptomycin–fungizone, 10 µg ml−1 insulin, 100 ng ml−1 cholera toxin, 20 ng ml−1 epidermal growth factor, 500 ng ml−1 hydrocortisone and 5% FBS at 37 °C in 5% CO2 humidified atmosphere. NIH-3T3 cells were grown in DMEM supplemented with PenStrep and 5% FBS at 37 °C in 5% CO2 humidified atmosphere.

Transfections of siRNA were done according to the manufacturer’s instructions. For 10 cm dishes, 750,000 cells were seeded and transfected 24 h later. For any other dish, cell numbers were adjusted to area. MTT assays were performed as previously described37.

Immunofluorescence. Cells were processed for immunofluorescence as described earlier38, but primary antibody incubation was overnight at 4 °C. Images were acquired in the Orbitrap analyser with resolution 60,000 at m/z 400.
Extended Data Figure 1 | Mass spectrometry of histone H2A.

a. Tandem mass spectrum of the unmodified peptide sequence VTIAQGGVLPNIQAVLLPK from histone H2A. The spectrum correlates with the Q104me modified spectrum presented in Fig. 1a. b. Isotope cluster of Q104me modified peptide VTIAQGGVLPNIQAVLLPK from H2A. Using high-resolution mass spectrometry ensures that peptides are identified with an accuracy of parts per million.
Extended Data Figure 2 | Position of H2AQ105 in the nucleosome.
Structure of the yeast nucleosome (1ID3). Q105 is highlighted as a red sphere, localized at the surface of the histone octamer without contacting the DNA, but it is part of the interface between H2A and H3. H3 is in yellow, H4 in orange, H2A in blue and H2B in green.
Extended Data Figure 3  |  Quality control of the H2A Q105me antibody.

a, Examples of dot blots of dilution series (1:2) of unmodified or methylated peptide spotted on 0.22 μm PVDF membrane and developed using the anti-H2A Q105me antibody. 

b, Left, western blot on lysates of cells harbouring either WT or Q105A H2A as sole source of histones; right, human lysate from MCF10A cells. All western blots were probed with the anti-H2AQ105me-specific antibody or anti-H2A upon stripping, respectively. 

c, Peptide competition by western blotting with the indicated peptides shows that only the methylated peptide can compete the signal of MCF10A lysates (peptide concentration was at 1 μg ml⁻¹). 

d, Direct enzyme-linked immunosorbent assay (ELISA) against the indicated peptides. Biotinylated peptides were immobilized on streptavidine-coated 96-well plates and the ELISA was performed using a 1:20,000 dilution of the anti-Q105me antibody. The ELISA was developed using horseradish peroxidase (HRP)-coupled secondary anti-rabbit antibody and 3,3′, 5,5′-tetramethylbenzidine (TMB) as substrate.
Extended Data Figure 4 | Western blot quantification of in vitro methylation reaction and detailed fragmentation table. a, Quantification of five independent in vitro methylation reactions. Western blots were scanned and intensities were determined using ImageJ. *P < 0.05. b, Theoretical and measured b- and y-ion fragment masses for Q104 methylation of human H2A (peptide sequence VTIAQGGVLPNIQAVLKP) for mass spectrum shown in Fig. 2e.
Extended Data Figure 5 | Western blot quantification and growth curves for Nop1/fibrillarin depletion. a, Quantification of western blots for Fig. 2f. Here, the average band intensities of three independent experiments were determined using ImageJ and plotted. The amounts of H2AQ105me were expressed as relative levels at time point 0. *P < 0.05. b, Growth curve of WT and nop1.3 ts strains upon shift to restrictive temperature. c, Alignment of yeast Nop1 and its mammalian orthologue fibrillarin indicates that both proteins share around 70% identity. d, Human fibrillarin is able to replace yeast Nop1 to methylate H2A Q105 in vivo. Note that human fibrillarin migrates slightly faster than yeast Nop1. e, Quantification of western blots for Fig. 2g. Here, the average band intensities of three independent experiments were determined using ImageJ and plotted. *P < 0.05. f, MTT assays of MCF10A cells transfected with scrambled RNA interference or RNA interference directed against fibrillarin (si5 and si6).
Extended Data Figure 6 | Deletion of rDNA copies does not affect H2AQ105me. The deletion of rDNA copies in yeast (as indicated in the figure) does not decrease the level of Q105me within the cells. Extracts from yeast isogenic yeast strains harbouring different numbers of rDNA repeats were subjected to western blotting and probed with the anti-Q105me and anti-H2A antibodies respectively.
Extended Data Figure 7 | Actinomycin D treatment leads to loss of Q104/5me in cells. a, Logarithmically growing yeast cells were treated with 500 ng ml$^{-1}$ actinomycin D (ActD$^+$) or dimethylsulphoxide (ActD$^-$) for 2 h before fixation. ChIP against Q105me and H2A was analysed with the indicated primer sets and the ratio treated/untreated (showing either an increase or decrease in treated/untreated samples) was plotted in log$_2$ scale. b, MCF10A cells were treated with either dimethylsulphoxide or 50 ng ml$^{-1}$ actinomycin D for 2 h specifically to shut down RNA polymerase-I-mediated transcription. Cells were fixed and processed for immunofluorescence with the indicated antibodies/dyes. c, As in b, but with NIH-3T3 cells.
Extended Data Figure 8 | Genetic interaction of spt16-11 and H2AQ105A.

a, Sequence of H2A with highlighted residues that genetically interact positively (light blue,17 or negatively (green)18 with FACT thermosensitive mutations. H2AQ105 (red) is part of this region. b, Analysis of wild-type H2A or H2AQ105A in either wild type (SPT16) or thermosensitive mutations (spt16-11) with respect to their effect on the induction of a FACT-dependent gene (GAL1). Cells were grown in Raffinose-containing media overnight, diluted into Raffinose-containing media and allowed to grow to mid-logarithmic phase before shifting them to restrictive temperature for 4 h. Gal1 was induced by addition of 2% galactose (final). Samples were taken before and 120 min after induction. The relative ratio of GAL1-mRNA levels of three independent experiments is given. Also note that this assay is artificial as Q105me is not detected at the GAL1 locus in wild-type strains and is only intended to show a potential genetic interaction between H2AQ105me and FACT if they occur at a given genomic location. It is important to note that GAL1 is an inducible gene and different functions of FACT are required for inducible and steadily expressed genes. The activation of promoters requires the removal of nucleosomes whereas constitutive transcription relies on both removal and re-deposition. To understand the differences between these two FACT functions, we looked at the steady-state level of (c) low, (d) mid- and (e) high constitutively expressed genes at 30°C. Interestingly, in contrast to inducible genes, many constitutive genes show increased transcription in Q105A and spt16, in agreement with a more open chromatin state.
Extended Data Figure 9 | FACT binds to a region spanning H2AQ105. a, Left, the peptides enriched in a phage display screen using recombinant Spt16/Pob3 as bait; right, a putative consensus binding sequence (red). b, The interaction between Q105 and FACT and the influence of its methylation are conserved from yeast to mammalian cells. HeLa nuclear extract was incubated with the indicated peptides bound to streptavidin-coupled Dynabeads. Bound proteins were analysed by western blotting.
Extended Data Figure 10 | Influence of H2AQ105A on Nap1 binding.

a, The induction kinetics for Gal1-driven H2A in wild-type and spt16 mutant cells, as well as H2AQ105A, is identical. Cells were treated as in Fig. 4f and samples were taken at the indicated time points and analysed by western blotting. b, H2A Q105 is not required for interaction with the histone chaperone Nap1. Flag-tagged Nap1 was used to immunoprecipitate either wild-type H2A or the Q105A mutant H2A. An untagged wild-type strain served as background control.