

# Chd1 chromatin remodelers maintain nucleosome organization and repress cryptic transcription

Bianca P. Hennig, Katja Bendrin, Yang Zhou & Tamás Fischer<sup>†</sup>

Biochemistry Center (BZH), Heidelberg University, Heidelberg, Germany

**Chromatin organization is essential for defining transcription units and maintaining genomic integrity in eukaryotes. In this study, we found that deletion of the *Schizosaccharomyces pombe* Chd1 chromatin remodelers, *hrp1* and *hrp3*, causes strong, genome-wide accumulation of antisense transcripts. Nucleosome mapping revealed a specific role for Chd1 remodelers in the positioning of nucleosomes in gene coding regions. Other mutations associated with enhanced cryptic transcription activity, such as *set2Δ*, *alp13Δ* and FACT complex subunit *pob3Δ*, did not, or only mildly, affect nucleosome positioning. These data indicate several mechanisms in the repression of cryptic promoter activity in eukaryotic cells.**

Keywords: Chd1; chromatin remodelers; cryptic transcription; nucleosome structure; *S.pombe*

EMBO reports (2012) 13, 997–1003. doi:10.1038/embor.2012.146

## INTRODUCTION

The eukaryotic genome is organized into a compact and elaborate chromatin structure. The basic units are the nucleosomes, consisting of 147-base pairs (bp) DNA wrapped around an octamer of histone proteins. Nucleosomes are further organized into higher-order structures that compact the genome and protect it against damage, but also limits the availability of the DNA to macromolecules. Chromatin-modifying activities can switch between open or closed chromatin conformations and allow regulated access to the DNA. The overall chromatin 'landscape' of a genomic region establishes specific functional regions, such as promoter or coding regions of transcriptional units. The definition of these units is not very strict, as demonstrated by extended transcriptional activity outside the canonical transcription units. A main source of such cryptic transcription activity is transcription initiation from cryptic promoters. As the DNA sequence requirements for RNA polymerase II (Pol II) initiation are rather loose and appear frequently in the genome, chromatin structure has a major role in distinguishing canonical promoters from cryptic promoters.

Promoter regions possess a particularly open chromatin structure, including nucleosome-free regions (NFRs) [1] and the surrounding hyper-acetylated nucleosomes [2]. The chromatin in coding regions is in a more closed conformation with regularly placed nucleosomes and low levels of histone acetylation [2]. The removal of acetyl marks from histones by histone deacetylase (HDAC) complexes is crucial for the maintenance of a closed chromatin conformation within coding regions. The evolutionarily conserved Rpd3S complex in *Saccharomyces cerevisiae* (*S. cerevisiae*) is responsible for histone deacetylation within gene coding regions [3,4]. Clr6 complex II is the *S. pombe* homologue of this complex [5]. Mutations in these complexes lead to increased histone acetylation levels in gene coding regions and activation of cryptic promoters [4,6]. The Set2 histone methyltransferase is also involved in repressing cryptic promoter activity. Set2 is recruited to the elongating Pol II complex and methylates histone H3 lysine 36 (H3K36me) in gene coding regions [7]. H3K36me is thought to be the recruitment signal for the Clr6 complex II through its chromodomain-containing subunit, Alp13 (Eaf3 in *S. cerevisiae*) [3,4,6]. However, recent findings revealed that localization of the HDAC complex to coding regions is unaffected in *set2Δ*, suggesting that H3K36me is mainly relevant for the activity of the HDAC complex [8].

The elongating Pol II also recruits complexes whose main activity is the reassembly of nucleosomes following transcriptional activity on the DNA. Among these, one of the best characterized is the FACT complex. Mutations in this complex lead to decreased histone occupancy levels in transcribed genes and increased activity of cryptic promoters in these regions [9–12]. Similarly, mutations in the transcription elongation factor Spt6 [9], or in the histone chaperone Asf1/HIR complex lead to decreased histone occupancy and increased activity of cryptic promoters [12–14]. Genome-wide screens also identified the chromatin remodelling enzymes Chd1 and Isw1 as factors required for repression of cryptic promoters in *S. cerevisiae* [12,15]. Chd1 was also found to interact with transcription elongation complexes such as the Paf and FACT complexes [15–17]. A recent study from the Owen–Hughes lab reported that Isw1, Isw2 and Chd1 are responsible for the positioning of evenly spaced nucleosomes in gene coding regions in *S. cerevisiae* [18]. *S. pombe* has a slightly different subset of chromatin remodelers than *S. cerevisiae*. While

Biochemistry Center (BZH), Heidelberg University, Im Neuenheimer Feld 328, Heidelberg 69120, Germany

<sup>†</sup>Corresponding author. Tel: +49 6221 544728; Fax: +49 6221 544369;

E-mail: [tamas.fischer@bzh.uni-heidelberg.de](mailto:tamas.fischer@bzh.uni-heidelberg.de)

*S. pombe* lacks the ISWI remodelers, *Isw1* and *Isw2*, it contains two Chd1 remodelers, *Hrp1* and *Hrp3*.

In this study, we attempted to identify factors that have a role in repressing cryptic transcription activity in *S. pombe*. We screened a deletion library for chromatin-related factors and tested their effect on cryptic transcription. We found that deletion of the *S. pombe* Chd1 chromatin remodelers, *hrp1* and *hrp3*, results in a marked increase in cryptic transcription. To determine the underlying molecular mechanism, we mapped genome-wide nucleosome position and histone acetylation patterns in the Chd1-deficient strain. These experiments uncovered a specific role for Chd1 remodelers in maintaining the highly ordered nucleosome structure within transcription units. We extended our analysis to other mutations also known to enhance cryptic transcription activity. Although these mutants accumulate cryptic transcripts very similarly to the Chd1-deficient strain, our data showed that the underlying mechanisms are remarkably different.

## RESULTS AND DISCUSSION

### Chd1-deficient cells accumulate cryptic RNA transcripts

To further understand the role of chromatin in defining transcription units, we sought to identify mutations that activate cryptic transcription. To monitor cryptic transcription, we quantified the levels of antisense (AS) transcripts for selected genes in the *S. pombe* genome. AS transcript levels are very low in wild-type (WT) strain but increase substantially in mutants causing cryptic transcription; therefore, they serve as a sensitive indicator of cryptic transcription. We isolated total RNA from deletion mutants of chromatin-related factors (*S. pombe* deletion library—Bioneer Corporation, Daejeon, South Korea) and performed strand-specific reverse transcription PCRs. As positive controls, we used strains lacking *Set2* and *Clr6* complex II subunit *Alp13*. Both mutants are known to accumulate cryptic transcripts [6]. Among the mutant strains that were tested, we observed that deletion of *hrp3* or *hrp1* also leads to increased levels of AS transcripts (Fig 1A). *Hrp1* and *Hrp3* are two members of the Chd1 subfamily of ATP-dependent chromatin remodelling factors in *S. pombe*. We also tested AS transcript levels in a *hrp1Δhrp3Δ* double-deletion strain (from herein also referred to as Chd1-deficient strain). The combination of the two deletions demonstrated a strong increase in the levels of the monitored AS transcripts (Fig 1A). Despite the strong AS transcript accumulation, the *hrp1Δhrp3Δ* strain did not demonstrate an obvious growth defect (Fig 1B). We also analysed genome-wide expression profiles in the above-mentioned mutant strains using high-resolution tiling microarrays (Fig 1C). The Chd1-deficient strain revealed strong, genome-wide AS transcript accumulation with 44% of the analysed genes showing significantly increased AS levels. Interestingly, changes observed in the sense transcripts were rather minor, with only 6% of the gene transcripts significantly up- or downregulated. Single deletions of *hrp3* or *hrp1* also showed moderate AS transcript accumulation (24% and 16%, respectively, of genes analysed). We also confirmed that deletion of *set2* or *alp13* leads to genome-wide AS transcript accumulation (40% and 56% of the genes, respectively) [6]. Comparison of these expression analyses revealed a remarkable similarity between the AS accumulation profile of the Chd1-, *Alp13*- and *Set2*-deficient strains (Fig 1C). This result suggests that Chd1-type chromatin remodelers, *Hrp1* and *Hrp3*, are responsible for the repression of cryptic promoter

activity in coding regions, similar to the previously characterized *Set2*–*Clr6* complex II pathway.

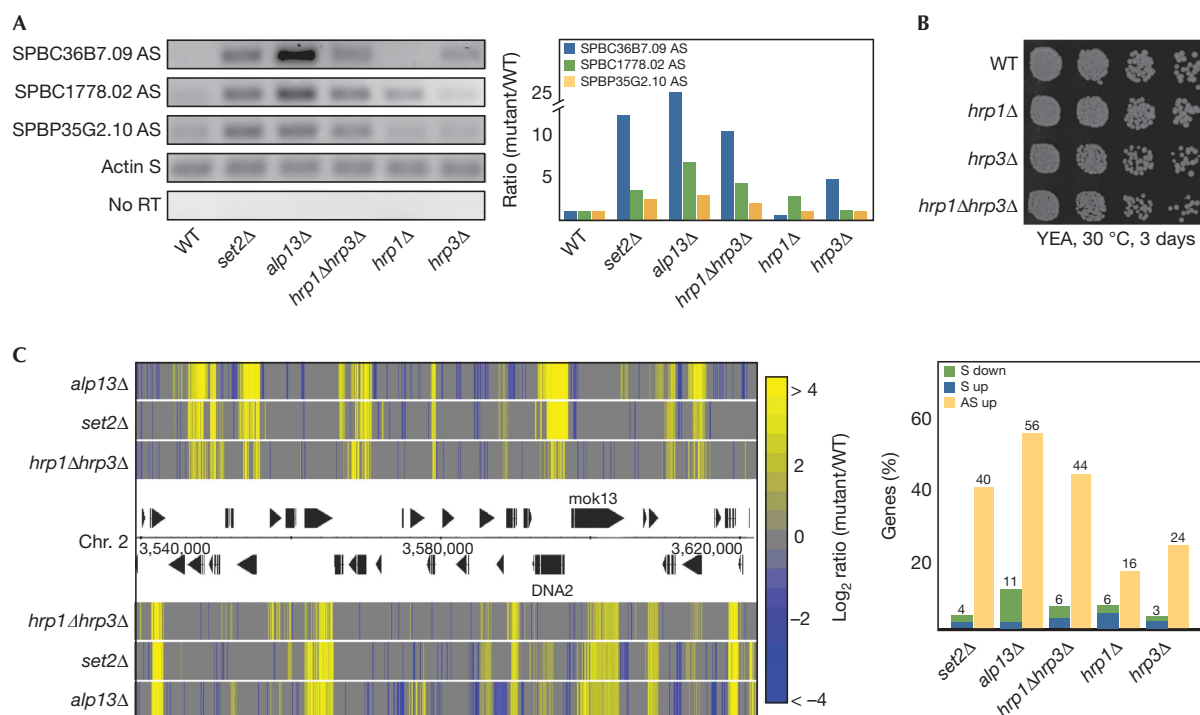
### Nucleosomes are not depleted in Chd1-deficient cells

Previous studies showed that genome-wide nucleosome depletion can activate cryptic transcription in yeast [12,14]. This prompted us to check nucleosome occupancy in the Chd1-deficient strain. Accurate quantification of nucleosome occupancy is very challenging because genome-wide techniques only provide relative enrichment values, and cannot detect changes affecting the whole genome. Instead, we determined total histone H3 levels in WT and mutant strains. As nearly all histones are incorporated into the chromatin, alteration of the total H3 content of a cell is a good indicator of changes in genome-wide nucleosome occupancy [19,20]. Western blot signals for bulk H3 levels from total cell extracts were quantified and normalized to the amount of total protein in the extract (Fig 2A; see signals normalized to actin in supplementary Fig S1A online). Quantification of western blot signals are limited in their precision and are dependent on the method of normalization. Therefore, our data are not a precise measurement but rather an indication of the changes in H3 content in the mutant strains compared with WT. Deletion of the FACT complex subunit *pob3* resulted in a significant decrease in bulk H3 levels, confirming previous findings that nucleosome occupancy is substantially decreased in FACT complex mutants [11,21]. In contrast, *alp13Δ*, *set2Δ* and *hrp1Δhrp3Δ* strains showed no significant change in their bulk H3 content. Although we cannot exclude a slight decrease in nucleosome occupancy in the *hrp1Δhrp3Δ* strain, a substantial nucleosome depletion such as the one observed in the *pob3Δ* strain was not detected in this mutant.

### ORFs are not hyper-acetylated in the *hrp1Δhrp3Δ* strain

As the pattern of AS transcript accumulation in *hrp1Δhrp3Δ* is very similar to those of *alp13Δ* and *set2Δ*, we wondered whether this similar phenotype might be the result of convergent molecular mechanisms. Current models suggest that both *set2* deletion and mutations in the *Clr6* complex II lead to increased histone acetylation in gene coding regions, resulting in enhanced cryptic promoter activity [4]. To test whether the increased cryptic transcription activity in the Chd1-deficient strain is also coupled to enhanced histone acetylation, we analysed bulk H3 acetylation levels in WT and mutant strains. We used a polyclonal antibody against acetylated H3 lysine 9 (H3K9ac) and H3 lysine 14 (H3K14ac), two known targets of the HDAC *Clr6* complex II. The signals were normalized to the H3 content of the strains (Fig 2B; normalization to total protein extract or actin in supplementary Fig S1B online). Similar to published results, bulk H3K9/K14ac levels were remarkably increased in the HDAC subunit deletion *alp13Δ* compared with the WT strain [5]. In contrast, we did not observe significant changes in *hrp1Δhrp3Δ*, indicating an HDAC-independent mechanism in the activation of cryptic transcription in this mutant. Remarkably, in the *set2Δ* strain we could detect only a moderate increase in bulk H3 acetylation.

To understand the acetylation pattern of the mutants in more detail, we performed chromatin immunoprecipitation (ChIP) experiments to map the genome-wide distribution of H3K9/K14ac in WT and mutant strains. Composite plot of H3K9/K14ac signals for 2,320 genes shows the characteristic acetylation



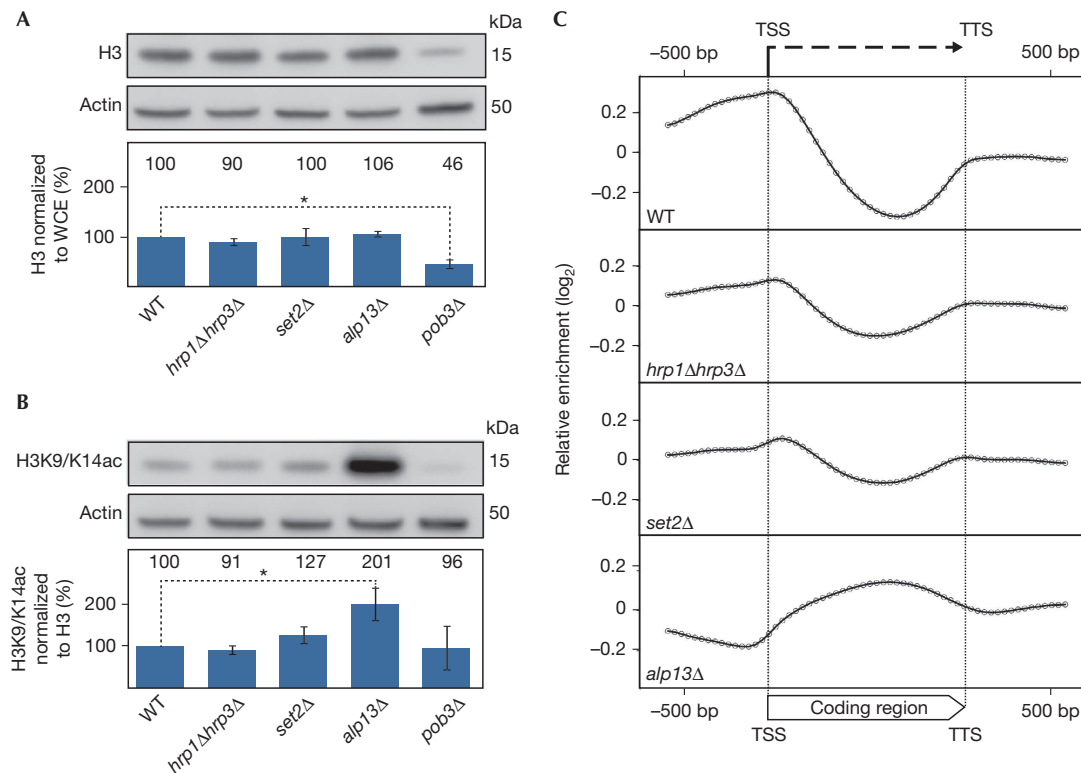
**Fig 1** | *hrp1Δhrp3Δ* cells accumulate AS transcripts. (A) Gel electrophoresis (left panel) of strand-specific reverse transcription and PCR amplification of the indicated S and AS transcripts in WT and mutant strains. Total RNA from the indicated strains was reverse transcribed using strand-specific primers, and the resulting complementary DNA was amplified by PCR. Controls lacking reverse transcriptase were used to exclude DNA contamination (no RT). The chart (right panel) indicates quantification of the PCR transcripts normalized to the amount of actin S transcripts (WT set to 1). (B) Growth of indicated strains in a series of fivefold dilutions on YEA plates. The cells were incubated at 30 °C for 3 days. (C) Heat map (left panel) showing a portion of the *S. pombe* genome demonstrating relative levels of transcripts from the forward (upper panel) and reverse (lower panel) directions in the indicated strains compared with WT. Colours represent up- or downregulation of gene expression on a log<sub>2</sub> scale. The chart (right panel) shows genome-wide statistics of changes in S and AS transcript levels for the indicated strains compared with WT. AS, antisense; Chr., chromosome; RT, reverse transcriptase; S, sense; WT, wild-type.

pattern in WT strain, with highly acetylated promoter regions and hypo-acetylated coding regions (Fig 2C). Deletion of *alp13* results in the redistribution of acetylated histones with dramatically increased levels in coding regions (Fig 2C). In contrast, the genome-wide distribution of acetylated histones in *hrp1Δhrp3Δ* and *set2Δ* is similar to WT, with hyper-acetylated promoter regions and hypo-acetylated coding regions (Fig 2C). The reduced ratio of acetylation levels in the promoter region to levels in the coding region might indicate a modest increase of acetylation in the coding regions, but can also be the consequence of generally decreased acetylation levels. This result further supports an HDAC-independent mechanism for activating spurious transcription in the Chd1-deficient strain. The unexpected finding that deletion of *set2*, the sole H3K36 methyltransferase in *S. pombe*, had a significantly different effect on H3 acetylation levels than deletion of *alp13* indicates that H3K36 methylation is not, or is only partly, responsible for the recruitment and activity of the HDAC Clr6 complex II in *S. pombe*.

### Hrp1 and Hrp3 organize nucleosomes in ORFs

As ATP-dependent chromatin remodelling enzymes are known to have a major role in the correct positioning of the nucleosomes, we decided to map nucleosome positions in WT and

Chd1-deficient strains. After a mild crosslinking and MNase treatment, we isolated and labelled the DNA from the mononucleosome fraction, and hybridized it to DNA microarrays covering 75% of the *S. pombe* genome at 10-bp resolution. Normalized hybridization signals were then used to determine the relative nucleosome occupancy levels. The lack of the linker histone H1 is compensated by very short linker DNA segments in *S. pombe*, which explains the median nucleosome distance of 150bp in the WT dataset, and it is in agreement with previously published results [22]. WT cells show the classical nucleosome organization pattern seen in *S. cerevisiae* and in higher eukaryotes: a pronounced NFR upstream of the transcriptional start site, flanked by the highly positioned +1 and -1 nucleosomes (Fig 3B; supplementary Fig S2A online), and an organized nucleosome array in the coding regions. This structure is particularly evident on the two-dimensional plot (Fig 3A), which reveals a remarkably regular nucleosome organization pattern in the entire coding region of the genes. In contrast, the Chd1-deficient strain showed disorganized nucleosome arrays in coding regions (Fig 3A,B). This effect is not the result of a significantly decreased number of nucleosomes, as the quantity of nucleosomes detected is only mildly reduced (-8%) compared with WT. The composite plot reveals that the organization of the NFR and the surrounding

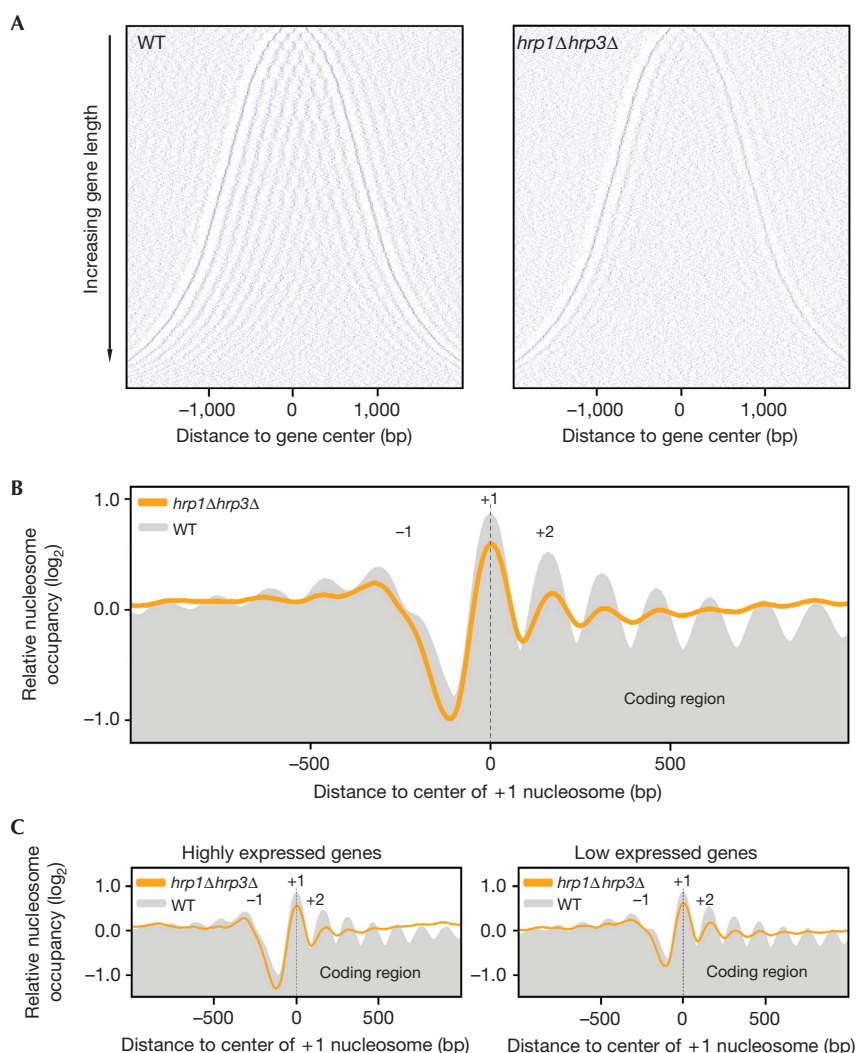


**Fig 2** | Coding regions are not hyper-acetylated in *hrp1Δhrp3Δ*. (A) WCE were subjected to SDS–polyacrylamide gel electrophoresis and H3 levels were monitored by western blot. The relative H3 levels were quantified and normalized to the total protein content of the WCE (chart, lower panel) and WT was set to 100%. (B) Western blot analysis of the same WCEs using a polyclonal antibody recognizing both acetylated H3K9 and H3K14 (H3K9/K14ac). The relative H3K9/K14ac levels were quantified and normalized to levels of H3, with WT set to 100% (chart, lower panel). For (A) and (B), data represent the means of three independent experiments and error bars represent s.d. \*Indicates statistical significance using Student’s *t*-test ( $P < 0.05$ ). (C) Composite plot of ChIP-on-chip analyses using the anti-H3K9/K14ac antibody in WT and indicated mutant strains. H3K9/K14ac relative enrichment values were determined in a genome-wide ChIP-on-chip experiment with 2,320 genes represented on the array. Each gene was divided into 30 parts (bins) and the average relative enrichment value was determined for each bin. In addition, 600 bp from both the flanking 5′ and 3′ regions were included in the analysis. The average of the log<sub>2</sub> values of each of these bins (geometric mean) was calculated and plotted on a log<sub>2</sub> scale, where each dot represents a bin. bp, base pairs; ChIP, chromatin immunoprecipitation; TSS, transcriptional start site; TTS, transcriptional termination site; WCE, whole-cell extracts; WT, wild-type.

+1 and −1 nucleosomes are similar to WT, but the subsequent peaks in the coding regions quickly disappear from the plot (Fig 3B). The resulting plateau in the composite plot is due to irregularly placed nucleosomes, which do not adhere to a position trend in the averaged dataset. We also detected a 10-bp increase in the median distance between nucleosomes. When nucleosome spacing is irregular, nucleosomes generally occupy a slightly longer DNA segment. A 10-bp increase in the average nucleosome distance would lead to the loss of roughly one nucleosome per gene for genes of average length. Extrapolating these numbers to the *S. pombe* genome would yield a ~6–8% loss in nucleosomes, which is compatible with our bulk H3 quantification results in this mutant. Our expression profiling experiments in the Chd1-deficient strain showed that the lack of an organized nucleosome structure in coding regions has only a minor effect on gene expression but results in significantly elevated cryptic transcription activity from coding regions. We also carried out nucleosome mapping in the *hrp1Δ* and *hrp3Δ* single-deletion strains (supplementary Fig S2B,C online). We could not detect a remarkable change in nucleosome

organization in the *hrp1Δ* strain compared to the WT. In contrast, *hrp3Δ* showed significantly disturbed nucleosome organization in coding regions, although this was less severe than in the *hrp1Δhrp3Δ* double-deletion strain. This suggests that the two Chd1-type chromatin remodeler enzymes, Hrp1 and Hrp3, have redundant functions in nucleosome organization in *S. pombe*.

Next, we analysed the effects of transcription levels on nucleosome organization in WT and Chd1-deficient strains. We sorted genes according to their expression levels, and plotted composite plots for the highest- or lowest-25% expressed genes (highly or lowly expressed genes) (Fig 3C). Both groups of genes were equally affected in the *hrp1Δhrp3Δ* strain, resulting in irregularly organized nucleosomes in the coding regions. We concluded that the activity of Hrp1 and Hrp3 is essential in the establishment of regularly spaced nucleosome arrays in coding regions independently of the transcriptional status of the gene. A recent study in *S. cerevisiae* found a similar role for Chd1 together with the ISWI chromatin remodelers Isw1 and Isw2. *S. pombe* does not possess ISWI chromatin remodelers, but two



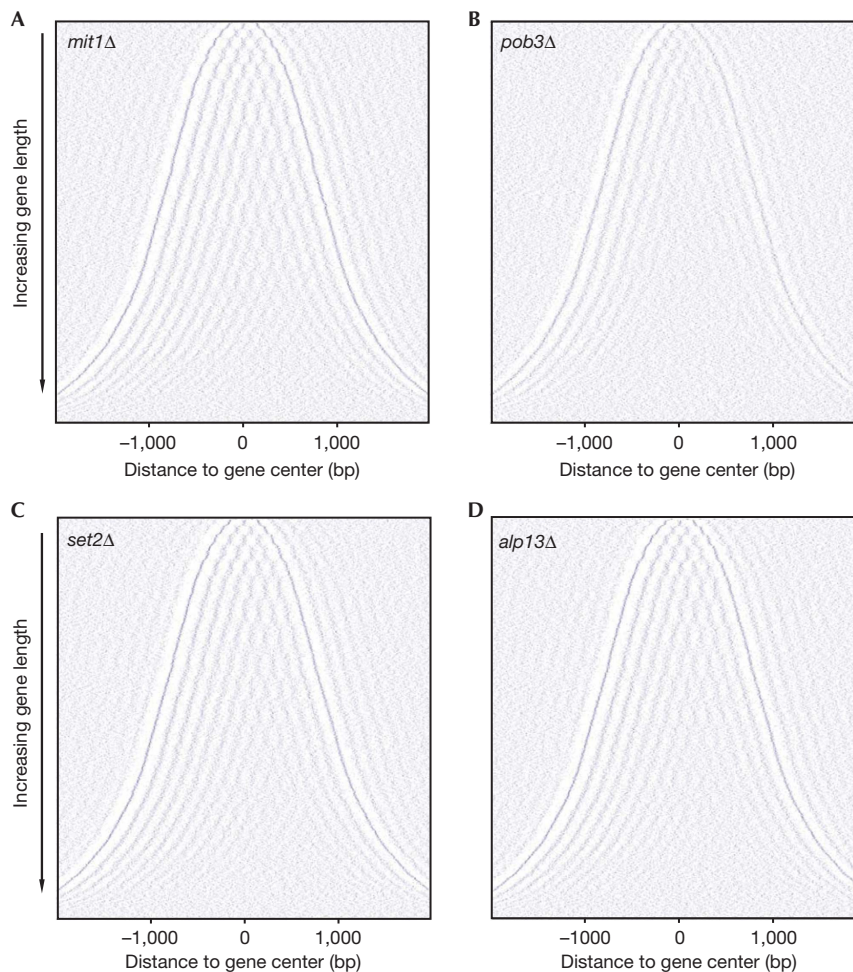
**Fig 3** | The regularly organized nucleosome arrays in gene coding regions are disrupted in the *hrp1Δhrp3Δ* strain. (A) Two-dimensional plots of nucleosomes along 3,778 genes in WT and *hrp1Δhrp3Δ* strains. Positions of the first and last nucleosomes were determined for each gene in an independent experiment with the WT strain. These annotations were used for the analysis of all data sets. Each row represents a gene; genes were sorted vertically (shortest at the top and longest genes at the bottom) according to the distance between the first and last nucleosome of the gene and were aligned at the mid point. Blue dots correspond to the centre of identified nucleosomes. (B) Composite plots of relative nucleosome occupancy for the WT (grey shading) and the *hrp1Δhrp3Δ* mutant (orange line). Using the same annotation from the independent WT data set, 3,778 genes were aligned at their first annotated nucleosome and the average of their log<sub>2</sub> nucleosome occupancy data (geometric mean) was plotted. (C) The genes represented in (B) were sorted according to gene expression levels, and composite plots were generated for the highest expressed 25% of genes (left) and the lowest expressed 25% of genes (right). bp, base pairs; WT, wild-type.

Chd1 remodelers. Considering that *S. pombe* and *S. cerevisiae* are evolutionarily very distant species, these results indicate an evolutionarily conserved role for Chd1 chromatin remodelers in the establishment of regularly spaced nucleosome structure in gene coding regions. Mit1, a member of the Chd3–Chd4 subfamily of chromatin remodelers, was also reported to function in the nucleosome organization of euchromatic gene coding regions [22]. Although we cannot exclude a minor effect, we did not observe significant changes in the chromatin structure of gene coding regions in *mit1Δ* (Fig 4A; supplementary Fig S3A online).

This underlines the specific role of the Chd1 chromatin remodelers in nucleosome organization in gene coding regions.

### Nucleosome arrays are mostly unaffected in *pob3Δ*

Mutations in the FACT complex can lead to significantly decreased histone levels and a concomitant reduction of genome-wide nucleosome occupancy [14,21]. Our western blot analysis confirmed substantial nucleosome depletion in the FACT complex subunit deletion strain, *pob3Δ* (Fig 2A). Nucleosome mapping experiments in *pob3Δ* showed that substantially



**Fig 4** | Two-dimensional plots of nucleosomes along 3,778 genes in the indicated mutant strains. Nucleosome positioning is unaffected in *mit1Δ* (A), *set2Δ* (C) and *alp13Δ* (D) strains, and is moderately affected in the *pob3Δ* strain (B). See Figure legend 3A for details. bp, base pairs.

decreased nucleosome occupancy had only a modest effect on nucleosome positions in gene coding regions (Fig 4B; supplementary Fig S3B online). We detected a mild, 10-bp increase in median nucleosome distance in gene coding regions, but the regularly organized nucleosome pattern remained mostly intact in the *pob3Δ* strain. Similar conclusions were presented by Celona *et al* [21] in a study investigating the deletion of the FACT complex subunit *Nhp6* in *S. cerevisiae* and its mammalian homologue, *Hmgb1*.

This result also suggests that the marked loss of regularly positioned nucleosomes in *hrp1Δhrp3Δ* is not a result of a reduction in the detection sensitivity of nucleosome positions due to decreased nucleosome occupancy levels. Nucleosome occupancy is not, or only mildly, reduced in the *hrp1Δhrp3Δ* strain, but the normal nucleosome positioning is diminished. In contrast, the *pob3Δ* strain exhibits strong nucleosome depletion, but only mildly irregular positioning of nucleosomes. Overall, these data indicate that the main role of the Chd1-type chromatin remodelers is to rearrange randomly positioned nucleosomes to equally spaced nucleosome arrays in gene coding regions.

### Nucleosome arrays in *alp13Δ* and *set2Δ* are unaffected

The AS transcript accumulation profile in the *hrp1Δhrp3Δ* strain is very similar to those of the *set2Δ* or *alp13Δ* strains. We ruled out the possibility that Hrp1 and Hrp3 function upstream of the Clr6 HDAC complex, as *hrp1Δhrp3Δ* did not show increased histone acetylation. An alternative possibility is that Hrp1 and Hrp3 function downstream of Set2 and the Clr6 complex II. In this hypothesis, *set2Δ* and/or *alp13Δ* strains would also show irregular nucleosome spacing in gene coding regions, similar to the Chd1-deficient strain. We could not detect significant changes in the nucleosome organization of these mutant strains compared to the WT. Two-dimensional plots (Fig 4C,D) and composite plots (supplementary Fig S3C,D online) of *set2Δ* and *alp13Δ* showed regularly positioned nucleosomes in coding regions. Although cryptic promoters are derepressed in these mutants, the nucleosome structure and occupancy are not affected. These results also indicate that Chd1 remodelers repress cryptic transcription independently of Set2 and the Clr6 complex II.

Our study shows that there are several parallel mechanisms responsible for the maintenance of a repressive chromatin

environment in gene coding regions. Disturbing any of these mechanisms results in activation of cryptic promoter sequences and accumulation of cryptic, non-coding RNAs. We showed that in the Chd1-deficient strain, nucleosomes are irregularly positioned in gene coding regions. We propose that the lack of organized nucleosome arrays can result in areas of nucleosome-free DNA, which can expose cryptic promoter sequences and lead to cryptic transcription. Other mutations, such as the deletion of the FACT complex subunit *pob3*, are associated with decreased nucleosome occupancy. Nucleosome positions are mostly unaffected in these mutants, but the time that these positions remain unoccupied is significantly longer. This temporarily allows transcription initiation events from exposed cryptic promoters. Interestingly, our study shows that deletion of the HDAC subunit *alp13* does not affect nucleosome positioning or occupancy, although cryptic transcription is derepressed. We speculate that in *alp13Δ*, the increased histone acetylation in coding regions recruits bromodomain-containing chromatin remodelling factors, such as the RSC complex, which might temporarily establish NFRs, thereby exposing cryptic promoter sequences. Nucleosome occupancy and positions were also unaffected in the *set2Δ* strain; however, levels of acetylation were significantly lower than in the *alp13Δ* strain. This suggests that there might be more, unknown mechanisms that are important in the repression of cryptic promoters in gene coding regions, and in the maintenance of genomic integrity.

## METHODS

See supplementary Table S1 online for a list of strains used in this study. Microarrays used for gene expression profiling contain alternating probes for both the forward and reverse DNA strand. This allows us to distinguish between sense and AS transcripts.

Western blot and ChIP experiments were performed using antibodies against histone H3 (ab1791, Abcam), H3K9/K14ac (pAB-005-044, Diagenode) and actin (MAB1501R, Millipore). All microarray experiments were repeated at least two times, with the exception of nucleosome mapping experiments with no detectable difference compared to the WT (*alp13Δ*, *set2Δ* and *mit1Δ*) and the single deletions *hrp1Δ* and *hrp3Δ*. Full methods and any associated references are available in the supplementary methods online.

**Accession codes.** The microarray data from this publication have been submitted to the Gene Expression Omnibus (GEO) database and assigned the following accession number: GSE40872.

**Supplementary information** is available at EMBO reports online (<http://www.emboreports.org>).

## ACKNOWLEDGEMENTS

We would like to thank Jutta Worsch for her excellent technical assistance and Emmalene Bartlett for proof reading the manuscript. This work was supported by a grant from the Ministry of Science, Research and the Arts of Baden-Wuerttemberg. Y.Z. was supported by China Scholarship Council (CSC).

**Author contributions:** B.P.H. and T.F. designed the experiments, analysed the data and wrote the manuscript. B.P.H., K.B. and Y.Z. performed the experiments.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## REFERENCES

- Jiang C, Pugh BF (2009) Nucleosome positioning and gene regulation: advances through genomics. *Nat Rev Genet* **3**: 161–172
- Wang Z, Zang C, Cui K, Schonnes DE, Barski A, Peng W, Zhao K (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* **5**: 1019–1031
- Keogh MC et al (2005) Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive rpd3 complex. *Cell* **4**: 593–605
- Carrozza MJ et al (2005) Histone H3 methylation by set2 directs deacetylation of coding regions by rpd3s to suppress spurious intragenic transcription. *Cell* **4**: 581–592
- Nakayama J, Xiao G, Noma K, Malikzay A, Bjerling P, Ekwall K, Kobayashi R, Grewal SI (2003) Alp13, an MRG family protein, is a component of fission yeast clr6 histone deacetylase required for genomic integrity. *EMBO J* **11**: 2776–2787
- Nicolas E, Yamada T, Cam HP, Fitzgerald PC, Kobayashi R, Grewal SI (2007) Distinct roles of HDAC complexes in promoter silencing, antisense suppression and DNA damage protection. *Nat Struct Mol Biol* **5**: 372–380
- Buratowski S, Kim T (2010) The role of cotranscriptional histone methylations. *Cold Spring Harb Symp Quant Biol* **75**: 95–102
- Govind CK, Qiu H, Ginsburg DS, Ruan C, Hofmeyer K, Hu C, Swaminathan V, Workman JL, Li B, Hinnebusch AG (2010) Phosphorylated Pol II CTD recruits multiple hdacs, including rpd3c(S), for methylation-dependent deacetylation of ORF nucleosomes. *Mol Cell* **2**: 234–246
- Kaplan CD, Laprade L, Winston F (2003) Transcription elongation factors repress transcription initiation from cryptic sites. *Science* **5636**: 1096–1099
- Mason PB, Struhl K (2003) The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. *Mol Cell Biol* **22**: 8323–8333
- Jamai A, Puglisi A, Strubin M (2009) Histone chaperone spt16 promotes redeposition of the original h3-h4 histones evicted by elongating RNA polymerase. *Mol Cell* **3**: 377–383
- Cheung V, Chua G, Batada NN, Landry CR, Michnick SW, Hughes TR, Winston F (2008) Chromatin- and transcription-related factors repress transcription from within coding regions throughout the *Saccharomyces cerevisiae* genome. *PLoS Biol* **11**: e277
- Yamane K, Mizuguchi T, Cui B, Zofall M, Noma K, Grewal SI (2011) Asf1/HIRA facilitate global histone deacetylation and associate with HP1 to promote nucleosome occupancy at heterochromatic loci. *Mol Cell* **1**: 56–66
- Keogh M-C (2012) The replication-independent histone H3-H4 chaperones HIR, ASF1, and RTT106 co-operate to maintain promoter fidelity. *J Biol Chem* **3**: 1709–1718
- Quan TK, Hartzog GA (2010) Histone H3K4 and K36 methylation, chd1 and rpd3s oppose the functions of *Saccharomyces cerevisiae* spt4-spt5 in transcription. *Genetics* **2**: 321–334
- Kelley DE, Stokes DG, Pery RP (1999) CHD1 interacts with SSRP1 and depends on both its chromodomain and its atpase/helicase-like domain for proper association with chromatin. *Chromosoma* **1**: 10–25
- Krogan NJ, Kim M, Ahn SH, Zhong G, Kobor MS, Cagney G, Emili A, Shilatifard A, Buratowski S, Greenblatt JF (2002) RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. *Mol Cell Biol* **20**: 6979–6992
- Gkikopoulos T, Schofield P, Singh V, Pinskaya M, Mellor J, Smolle M, Workman JL, Barton GJ, Owen-Hughes T (2011) A role for snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization. *Science* **6050**: 1758–1760
- Gunjan A, Paik J, Verreault A (2006) The emergence of regulated histone proteolysis. *Curr Opin Genet Dev* **2**: 112–118
- Feser J, Truong D, Das C, Carson JJ, Kieft J, Harkness T, Tyler JK (2010) Elevated histone expression promotes life span extension. *Mol Cell* **5**: 724–735
- Celona B et al (2011) Substantial histone reduction modulates genomewide nucleosomal occupancy and global transcriptional output. *PLoS Biol* **6**: e1001086
- Lantermann AB, Straub T, Strålfors A, Yuan GC, Ekwall K, Korber P (2010) *Schizosaccharomyces pombe* genome-wide nucleosome mapping reveals positioning mechanisms distinct from those of *Saccharomyces cerevisiae*. *Nat Struct Mol Biol* **2**: 251–257