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Authors

Anver, Shajahan
Roguev, Assen
Zofall, Martin
et al.

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Yeast X-chromosome-associated protein 5 (Xap5) functions with H2A.Z to suppress aberrant transcripts

Shajahan Anver[1,†], Assen Roguev[2], Martin Zofall[3], Nevan J Krogan[2], Shiv I S Grewal[3] & Stacey L Harmer[1,*]

Abstract

Chromatin regulatory proteins affect diverse developmental and environmental response pathways via their influence on nuclear processes such as the regulation of gene expression. Through a genome-wide genetic screen, we implicate a novel protein called X-chromosome-associated protein 5 (Xap5) in chromatin regulation. We show that Xap5 is a chromatin-associated protein acting in a similar manner as the histone variant H2A.Z to suppress expression of antisense and repeat element transcripts throughout the fission yeast genome. Xap5 is highly conserved across eukaryotes, and a plant homolog rescues *xap5* mutant yeast. We propose that Xap5 likely functions as a chromatin regulator in diverse organisms.

Keywords aberrant transcripts; chromatin modification; long terminal repeats; transcriptional repression; transposable elements
Subject Categories Chromatin, Epigenetics, Genomics & Functional Genomics; Transcription
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Introduction

Global regulation of transcription is a key for organismal success, especially under stressful environmental conditions [1,2]. Repression of aberrant transcripts such as transposons, long terminal repeats (LTRs), other repeat elements, and antisense transcripts is as vital for success as the regulation of protein-coding genes [3–5]. The accessibility of DNA to the transcriptional machinery is controlled by its association with histones and other chromatin proteins. Chromatin properties, and therefore transcription, are in large part determined by posttranslational modifications of histones and the incorporation of variant histones in nucleosomes [6]. A genetic means of identifying previously unknown chromatin-associated genes is the epistatic miniarray profile (E-MAP) method [7,8]. We recently carried out an E-MAP experiment assessing the genetic interactions of *X-chromosome associated protein 5* (*xap5*) with approximately half of the genes encoded by the *Schizosaccharomyces pombe* genome.

Xap5-like proteins are highly conserved across eukaryotes [9]. These proteins are nuclear localized in yeast and plant cells [9,10]. The human *XAP5* gene is located in the Xq28 region, where many disease genes map [11] and has trinucleotide CCG repeats in the 5′ UTR, leading to the speculation that XAP5 may be implicated in human disease [12]. In addition, the paternally imprinted autosomal paralog of human *XAP5*, *XAP5-like* is located at 6p25.2 region and may play a role in spermatogenesis and carcinogenesis [13]. However, the cellular function of Xap5 proteins has not been described in any organism. We now report that the *S. pombe* and *Arabidopsis thaliana* Xap5-like proteins are functionally conserved and Xap5 is a novel, evolutionarily conserved chromatin-binding protein that affects chromatin regulation in a manner similar to the variant histone H2A.Z.

Results and Discussion

Xap5 function is conserved between widely diverged eukaryotes

In order to characterize the function of a Xap5-domain protein in a simple eukaryote, we obtained *S. pombe* with a deletion in *xap5* (SPCC1020.12c). In minimal media (EMM50), Δ*xap5* grows slightly more slowly than wild type at 30°C and this is exacerbated under nonoptimal growth temperatures (Fig 1A and B and Supplementary Fig S1). Xap5 family proteins are highly conserved across eukaryotes, suggesting that they may have similar molecular functions in diverse organisms [9]. We therefore tested whether the *Arabidopsis thaliana xap5* homolog *XCT* could rescue *S. pombe* mutant for *xap5*. The growth kinetics of wild-type, Δ*xap5* mutants transformed with either plant *XCT* (Δ*xap5-XCT*) or *S. pombe xap5* (Δ*xap5-xap5*) grown at 21°C, are virtually

1 Department of Plant Biology, College of Biological Sciences, University of California, Davis, CA, USA
2 Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA
3 Laboratory of Biochemistry and Molecular Biology, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA
*Corresponding author. Tel: +1 530 752 8101; E-mail: slharmer@ucdavis.edu
†Current address: Max Plank Institute for Plant Breeding Research, Cologne, Germany

indistinguishable (Fig 1A and B). Indeed, the generation time is almost twofold longer for the Δ*xap5* mutant than the *XCT* and *xap5* transformants (Fig 1A and B).

Given that Arabidopsis and fission yeast diverged more than 1 billion years ago [14] and that these two Xap5 homologs show the lowest levels of amino acid sequence conservation among the examined taxa with an identifiable Xap5 homolog [9], our findings suggest that Xap5 proteins are functionally well conserved across eukaryotes, sharing common molecular roles in diverse eukaryotes. A better understanding of the role of Xap5 in fission yeast will therefore likely provide insight into the function of Xap5 homologs across eukaryotes.

xap5 genetically interacts with known chromatin-associated genes

We next performed a high-throughput genetic interaction analysis in *S. pombe* to obtain evidence regarding pathways in which *xap5* might function. Only a few mutants have significant genetic interactions with Δ*xap5* at 30°C, but many do when yeast are grown at suboptimal temperatures of 20 or 37°C (Supplementary Dataset S1). Out of the 2,117 tested, we found that 324 mutants have significant genetic interactions with *xap5* at 37°C and 172 at 20°C. Importantly, there is considerable overlap between the significantly interacting mutants at these two temperatures (Fisher's exact test, $P < 2.2e\text{-}16$) and the gene ontology (GO) distributions for biological process and the genetic interaction score (S-score) profiles are also similar (Supplementary Fig S2). To identify pathways in which Xap5 might act, we looked for enrichment of particular GO terms among mutants that significantly interact with Δ*xap5* or that have similar genetic interaction profiles. Mutants that positively interact with Δ*xap5* at 37°C are enriched for histone modification (GO ID 16570, $P = 0.035$) and covalent chromatin modification (GO ID 16569, $P = 0.035$) functions.

In addition, of the 953 mutants (872 loci) previously subjected to E-MAP analysis, those with similar genetic interactions as Δ*xap5* (positively correlated genes; Supplementary Dataset S1) are enriched for roles in chromatin modification-mediated transcriptional control (Supplementary Table S1, Fig 1C). Individual mutants with genetic interaction profiles similar to Δ*xap5* include genes that function in well-known chromatin remodeling protein complexes including multiple members of both the Set1C/COMPASS complex, which is involved in H3K4 methylation, and the Swr1C complex, which catalyzes the deposition of the histone variant H2A.Z (encoded by the *pht1* gene) (Fig 1C–E, Supplementary Table S2). Genes that act in similar pathways often have positively correlated genetic interaction profiles [15], suggesting that Xap5 is involved in chromatin regulation or maintenance. Since multiple Pht1/H2A.Z mutants are significantly correlated with Δ*xap5*, we compared genome-wide gene expression profiles of yeast with deletions in either *xap5* or *pht1*, as described in more detail below. The Δ*pht1* and Δ*xap5* mutants have well-correlated gene expression profiles (Fig 1F), indicating that Xap5 and Pht1/H2A.Z regulate similar loci. Together, the genetic and gene expression data strongly suggest that Xap5 plays a role in chromatin modification.

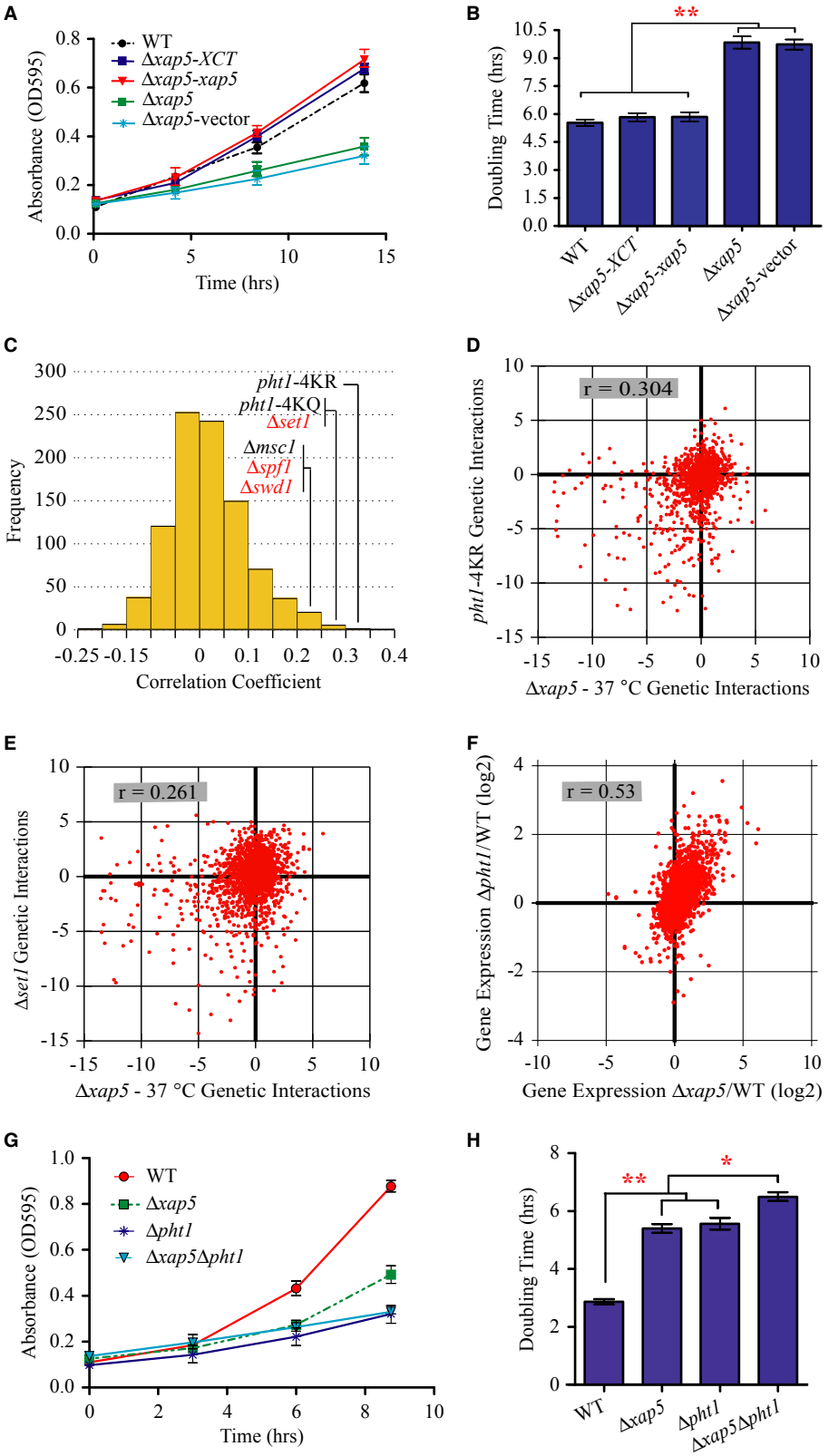
We wished to confirm the genetic interactions between Δ*xap5* and selected chromatin mutants with positively correlated genetic interactions (Supplementary Table S2). Like Δ*xap5* mutants, Δ*pht1* mutants have a subtle slow-growth phenotype in EMM50 at 30°C that is aggravated at 37 and 20°C and exacerbated when this mutant is combined with the Δ*xap5* mutation (Fig 1G and H and Supplementary Fig S3). Like *pht1*, *set1* (which encodes an H3K4 methyltransferase) is also statistically significantly genetically correlated with *xap5* (Fig 1C and E). However, there is an antagonistic genetic interaction between the *set1* and *xap5* mutants (Supplementary Fig S3E). Together, these data suggest that Xap5 plays a role in chromatin modification, acting in a similar pathway to Pht1/H2A.Z and antagonistically with Set1.

Antisense, intergenic, and other noncoding transcripts are misexpressed in the absence of Xap5

Since both Set1 and Pht1/H2A.Z play roles in the regulation of transcription, we carried out RNA-seq analysis to characterize the transcriptome of Δ*xap5* mutants. As previously reported for Δ*pht1* [16], we found that hundreds of loci corresponding to multiple types of transcripts are misregulated in both Δ*xap5* and Δ*pht1* (Supplementary Dataset S2; Fig 2A) and that the transcriptomes of Δ*xap5* and Δ*pht1* mutants are positively correlated (Fig 1F). The Arabidopsis *XCT* gene almost completely rescues the misregulation of gene expression in Δ*xap5* (Fig 2A and B), consistent with its ability to rescue the Δ*xap5* growth phenotype (Fig 1A and B). The Δ*xap5*Δ*pht1* double mutant displays a synergistic increase in all types of misregulated transcripts relative to either single mutant (Fig 2A and B), consistent with Xap5 and Pht1/H2A.Z playing similar but not identical roles. The misregulated loci include protein-coding (both sense and antisense), intergenic, and other noncoding sequences. Notably, a higher fraction of the misexpressed loci in both Δ*xap5* and Δ*pht1* correspond to antisense, intergenic, or other noncoding transcripts than to sense protein-coding transcripts (Fig 2A and B).

More than 80% of the loci significantly misregulated ($P \leq 0.01$, fold change ≥ 2) in Δ*xap5*, including both coding and noncoding transcripts, are upregulated (Fig 2C–H and Supplementary Fig S4A–H). Upregulated genes are significantly enriched for GO categories involved in stress responses (Supplementary Table S3), whereas downregulated genes are enriched for diverse biological processes including translation and processing of noncoding RNAs (Supplementary Table S4). The temperature-dependent slow-growth phenotypes and upregulation of stress-related transcripts in *xap5* mutants indicate that Xap5 function is especially important in adverse environmental conditions. As expected given the positive correlation between the transcriptomes of the Δ*xap5* and Δ*pht1* mutants (Fig 1F), many loci upregulated in Δ*xap5* are also upregulated in Δ*pht1* and Δ*xap5*Δ*pht1* (Fig 2C–H and Supplementary Fig S4A–H). Centromeres, telomeres and silent mating-type loci are not expressed in either the Δ*xap5* or the Δ*pht1* mutant, indicating that Xap5 and Pht1/H2A.Z are not essential for maintenance of strongly heterochromatic regions. Consistent with this, neither Xap5 nor Pht1/H2A.Z [16] association with regions of constitutive heterochromatin is enriched relative to the rest of the genome (Supplementary Dataset S3).

Antisense transcripts are prominently upregulated in Δ*xap5* and Δ*pht1* and are synergistically overexpressed in Δ*xap5*Δ*pht1* (Fig 2A and B). There are different patterns of correlation between the sense and the antisense transcripts of individual genes suggesting that the



misregulation of sense transcripts in Δ*xap5* is not primarily due to misregulation of antisense transcripts (Supplementary Fig S4). Many (54%) of the antisense transcripts in Δ*xap5* and more than 90% of the antisense transcripts in Δ*pht1* [16] map to convergent loci, suggesting these may have resulted from inappropriate read-through transcription (Fig 2I). We confirmed that this is the case for one

Figure 1. Δ*xap5* genetic interaction profile is correlated with that of known chromatin modifiers.

A, B Yeast Δ*xap5* mutants grown in EMM50 at 21°C exhibit a slow-growth phenotype rescued by the Arabidopsis *XCT* gene. Mean ± SEM of at least three independent experiments with at least three replicates ($n \geq 3$).

C Frequency distribution plot of correlation coefficients of the *xap5* mutant genetic interaction profile with that of other mutants. Red = members of Set1C/COMPASS and black = members of Swr1C. In the two *pht1* mutant alleles shown, the four lysines (4K) at the N-terminus were mutated to unacetylatable residues, disrupting their function [32].

D, E *pht1* (D) and *set1* (E) mutant genetic interaction (S-score) profiles versus that of the *xap5* mutant (Pearson, $P < 2.2e\text{-}16$).

F Transcriptome profiles of Δ*xap5* versus Δ*pht1* (Spearman, $r = 0.53$, $P < 2.2e\text{-}16$).

G, H *H2A.Z/pht1* genetically interacts with *xap5*. Growth curves for Δ*xap5*, Δ*pht1*, and Δ*xap5*Δ*pht1* mutants in EMM50 at 37°C.

Data information: Mean ± SEM of at least three independent experiments with at least three replicates ($n \geq 3$). In (B) and (H): one-way ANOVA with Bonferroni's multiple comparison test, $*P = 0.05$, $**P = 0.0001$.

locus using strand-specific RT–PCR (Fig 2J). Consistent with a general role for read-through transcription in the generation of antisense transcripts, we observed more upregulation of antisense transcripts near transcription termination sites than near transcription start sites (Fig 2K, Supplementary Methods). Of the 4,240 detectably expressed genes, 178 and 113 display faulty transcriptional termination in Δ*xap5* and Δ*pht1* mutants, respectively (Supplementary Dataset S2; see Supplementary Methods for analysis details). Thirty-eight of these inappropriately terminated transcripts are shared between the two single mutants (significantly more overlap than expected by chance; $P = 6.7e\text{-}15$, Fisher's exact test). This suggests that Xap5 is somehow required for proper transcription termination. Further strengthening this possibility, as described in more detail below, we found that Xap5 protein is preferentially associated with the gene bodies and transcription termination sites of loci with faulty transcription termination in Δ*xap5* mutants (Fig 4E).

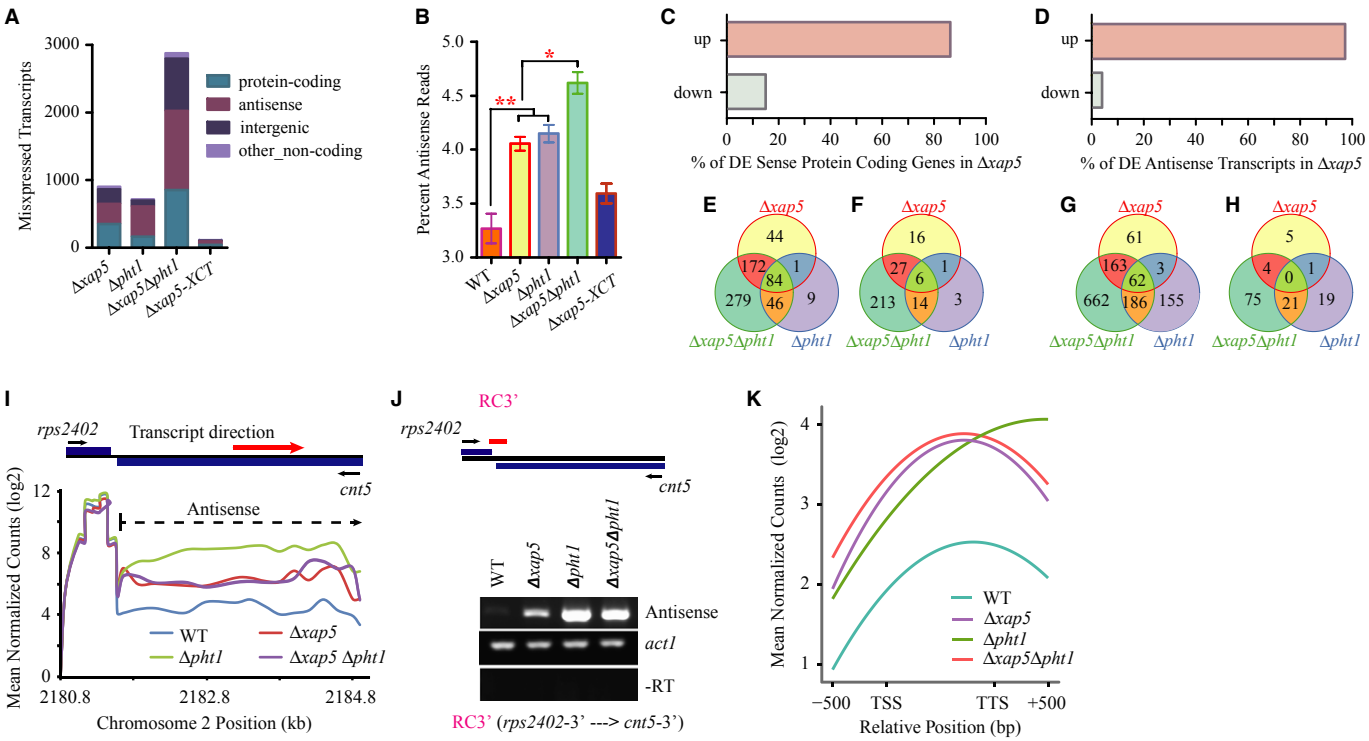


Figure 2. Aberrant transcripts are upregulated in Δ*xap5*.

A Types of transcripts differentially expressed in the indicated genotypes.

B Percent total antisense reads (antisense reads as a fraction of total reads per library) in the mutants. One-way ANOVA with Bonferroni's multiple comparison test, $*P = 0.05$, $**P = 0.03$, mean ± SEM of two lanes with three biological replicates ($n = 3$).

C, D Percentage of protein-coding gene (C) sense and (D) antisense transcripts up- or downregulated in Δ*xap5*.

E–H Venn diagrams showing the overlap between (E) upregulated and (F) downregulated sense protein-coding genes and (G) upregulated and (H) downregulated antisense transcripts in the indicated mutants.

I Read-through transcription at the *rps2402-cnt5/csx2* locus generates antisense transcripts identified by RNA-seq.

J Strand-specific RT–PCR confirms the presence of a read-through transcript (RC3′). Similar results were observed at least in two independent experiments.

K Genome-wide abundance of antisense transcripts is significantly higher in all three mutants at transcription termination sites (TTS) than transcription start sites (TSS) (Welch's two-sample *t*-test, $P = 0.002$). The values are the means of three biological replicates processed in parallel ($n = 3$). Statistical significance of differential expression was determined using the negative binomial exact test ($P \leq 0.01$, fold change ≥ 2).

Spurious antisense reads can be generated during library synthesis due to second-strand cDNA synthesis during reverse transcription, an event that can be inhibited by the addition of actinomycin D [17], albeit at the expense of decreased 5′ and 3′ coverage of transcripts and increased variation in overall expression levels [18]. Although we did not add actinomycin D during library synthesis, the reproducible detection of increased antisense levels in the mutants relative to wild type (Fig 2B), the inverse correlations between changes in expression for many sense and antisense transcripts (Fig S4I and J), and our verification of upregulation of many antisense transcripts using locus-specific primers (Fig 2J, Supplementary Figs S4G and S5C) supports our conclusion that Xap5 and Pht1/H2A.Z cooperate to suppress expression of a variety of aberrant sequences including antisense and read-through transcripts at loci throughout the *S. pombe* genome. Indeed, the Δ*xap5*Δ*pht1* double mutants display an enhanced increase in antisense transcripts (Fig 2B) very similar to that seen when the Δ*pht1* mutant is combined with mutations in several different silencing complexes [16].

Xap5 and H2A.Z/Pht1 bind chromatin in repeat element loci to repress their transcription

Along with upregulation of protein-coding transcripts, many repeat elements, including retrotransposons (*Tf2* elements), LTRs, and *wtf* elements are upregulated in Δ*xap5*, Δ*pht1,* and Δ*xap5*Δ*pht1* (Fig 3). Out of the 238 LTRs annotated in the *S. pombe* genome, 117 are detectably expressed in wild type and all three mutants (Supplementary Dataset S2). Of these 117 LTRs, 21 (~18%) and 28 (24%) are significantly ($P \leq 0.05$) upregulated in Δ*xap5* and Δ*pht1*, respectively. This number increases synergistically to 79 (~67.5%) in the double mutant (Fig 3A and B). Of 25 *wtf*s in the *S. pombe* genome, 17 and 22 are significantly ($P \leq 0.01$) upregulated in the Δ*xap5* and Δ*xap5*Δ*pht1* mutants, respectively (Fig 3C and D and Supplementary Fig S4H). Relative to all genes, these different types of repeat loci are preferentially upregulated in both Δ*xap5* and Δ*pht1* mutants (Fisher's exact test, *P*-values for LTRs = 1.203e-05 and *wtf*s < 2.2e-16 in Δ*xap5* and LTRs < 2.2e-16 and *wtf*s = 0.006443 in Δ*pht1*). This pattern suggests that Xap5 and Pht1/H2A.Z are involved in silencing these repeat elements.

Since Xap5 affects processes regulated by known chromatin-associated proteins, we investigated whether Xap5 itself binds chromatin using chromatin immunoprecipitation followed by microarray analysis (ChIP-chip). We found that Xap5 protein is associated with chromatin in both genic and intergenic regions (Supplementary Dataset S3). Both Xap5 and Pht1/H2A.Z are significantly enriched at all 13 *Tf2* transposable element loci (Fig 4A and B). Consistent with these associations having functional significance, *Tf2* ORFs are upregulated in both Δ*xap5* and Δ*pht1* mutants (Figs 3E and 4B and Supplementary Table S5). Many LTRs and *wtf*s are significantly enriched for association of both Xap5 and H2A.Z (Fig 4A and C), and these and adjacent loci have altered expression in both mutants (Fig 4C, Supplementary Table S5 and Fig 3A–D).

We next investigated whether Xap5 and Pht1/H2A.Z association with chromatin is interdependent. We found no significant alteration of Pht1/H2A.Z binding to chromatin in Δ*xap5* mutants and no global change in Xap5 association with chromatin in Δ*pht1* mutants (Fig 4D and Supplementary Dataset S3). However, we found an appreciable increase in Xap5 association with *Tf2* and *wtf* loci in Δ*pht1* mutants relative to wild type (Fig 4D, Supplementary Dataset S3). These observations suggest Xap5 and Pht1 cooperatively contribute to the repression of transcription of repeat elements such as *wtf*s and *Tf2*s, such that loss of both genes causes synergistic upregulation of expression of these loci.

Similar upregulation of cryptic transcripts including repeat elements has previously been observed in silencing and RNAi pathway mutants in fission yeast [4,19,20] and chromatin regulatory gene mutants in budding yeast [21–23]. Moreover, the combination of such mutants with Δ*pht1* has previously been shown to synergistically increase expression of antisense RNAs in fission yeast [16]. We therefore compared the Δ*xap5* transcriptome with previously published microarray data for several gene-silencing mutants (*clr6.1, clr4.681,* and *clr6.1Δclr3*) and one RNAi mutant (Δ*dcr1*) [4]. We found moderate but statistically significant levels of correlation between the transcriptomes of Δ*xap5* and all these mutants, with the highest correlation seen between Δ*xap5* and *clr6.1Δclr3,* which have mutations in genes encoding histone deacetylases (Supplementary Fig S5A and B). These correlations might well be higher if not for the differences in growth conditions, gene expression analysis platforms, and library construction methods between the experiments being compared [4]. Since many types of noncoding transcript loci are upregulated in different histone deacetylase complex mutants [24], we examined the expression of several antisense and intergenic loci that are misexpressed in Δ*xap5* and Δ*pht1* in five different histone deacetylase complex mutants including Δ*clr3* and *clr6-1*. Strand-specific RT–PCR revealed that these loci are upregulated in all of these mutants as well as in Δ*xap5* and Δ*pht1* (Supplementary Fig S5C).

Though the H3K9 methyltransferase Clr4, the histone deacetylases Clr6 and Clr3, and the RITS component Dcr1 are best known for their roles in silencing of heterochromatic regions, these proteins are also involved in the regulation of euchromatic loci [4,19,25]. Many genes involved in environmental stress responses are derepressed in the *clr* mutants [4], similar to what we observe in Δ*xap5*. In addition, the above Clr proteins are required for the silencing of retrotransposons, LTRs, and *wtf*s [4,19]; notably, these types of loci are also enriched for Xap5 association and are derepressed in Δ*xap5*. Moreover, Clr6 is a component of the Rpd3L complex [26], other members of which have significantly correlated genetic interactions with *xap5* (Supplementary Table S2). Therefore, overall these correlations further support our conclusion that Xap5 cooperates with well-studied protein complexes to regulate chromatin.

Conclusions

Xap5 proteins lack recognizable functional domains, making prediction of their molecular functions difficult [9,12]. However, our genome-wide investigation has allowed us to assign a deeply conserved role for Xap5 in the regulation or maintenance of euchromatin. Notably, hundreds of loci are misregulated in the Δ*xap5* mutant (Fig 2), indicating that it is indeed required for appropriate transcriptional regulation across the *S. pombe* genome. However, Xap5 is not likely to encode a typical transcription factor: most misexpressed sequences in Δ*xap5* are upregulated, and among these 60% represent aberrant transcripts such as antisense and intergenic sequences while only 36% correspond to protein-coding gene

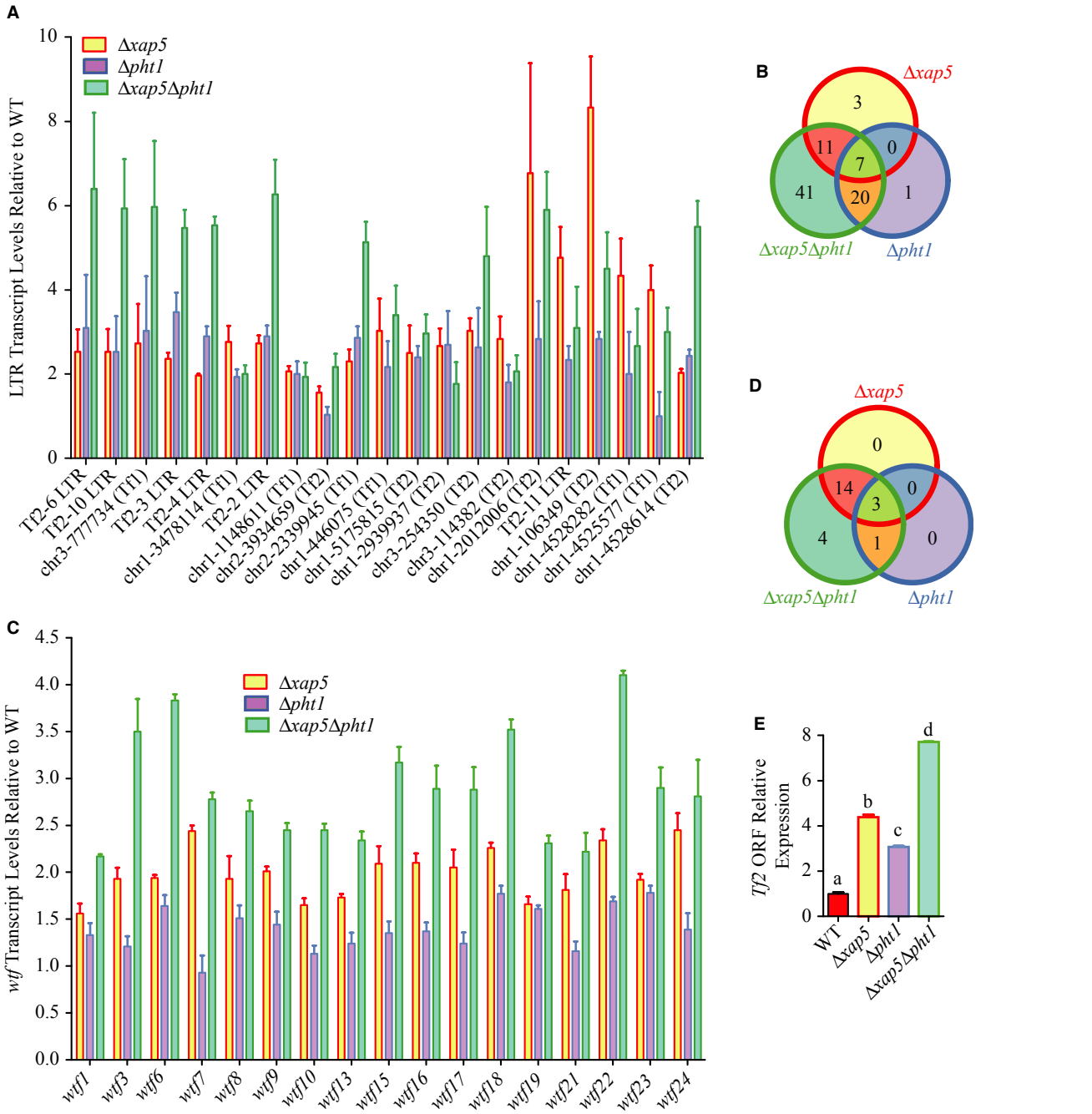


Figure 3. Many repeat elements are significantly upregulated in the *Δxap5* mutant.

A, B LTRs significantly upregulated ($P \leq 0.05$) in the Δ*xap5* mutant and (B) overlap between upregulated LTRs among different genotypes. Mean ± SEM of three biological replicates processed in parallel ($n = 3$).

C, D *wtf* elements significantly upregulated ($P \leq 0.01$) in the Δ*xap5* mutant and (D) the overlap between upregulated *wtf*s among different genotypes. Mean ± SEM of three biological replicates processed in parallel ($n = 3$).

E *Tf2* transposable element ORFs are upregulated in the mutants (quantitative RT–PCR, letters denote statistically significantly different groups, one-way ANOVA with Bonferroni's multiple comparison test, $P < 0.01$, mean ± SEM of three biological replicates processed in parallel ($n = 3$); experiment was repeated twice with similar results).

transcripts (including the *wtf*s, a family of genes of unknown function). Therefore, the primary function of Xap5 appears to be the genome-wide transcriptional silencing of antisense and intergenic and repeat element loci. Several distinct classes of factors have previously reported to be involved in such repression, including H2A.Z, the RNAi machinery, chromatin remodelers, histone methyltransferases, and the exosome [16,21,22,25,27–29]. A precise determination of the molecular function of the conserved chromatin

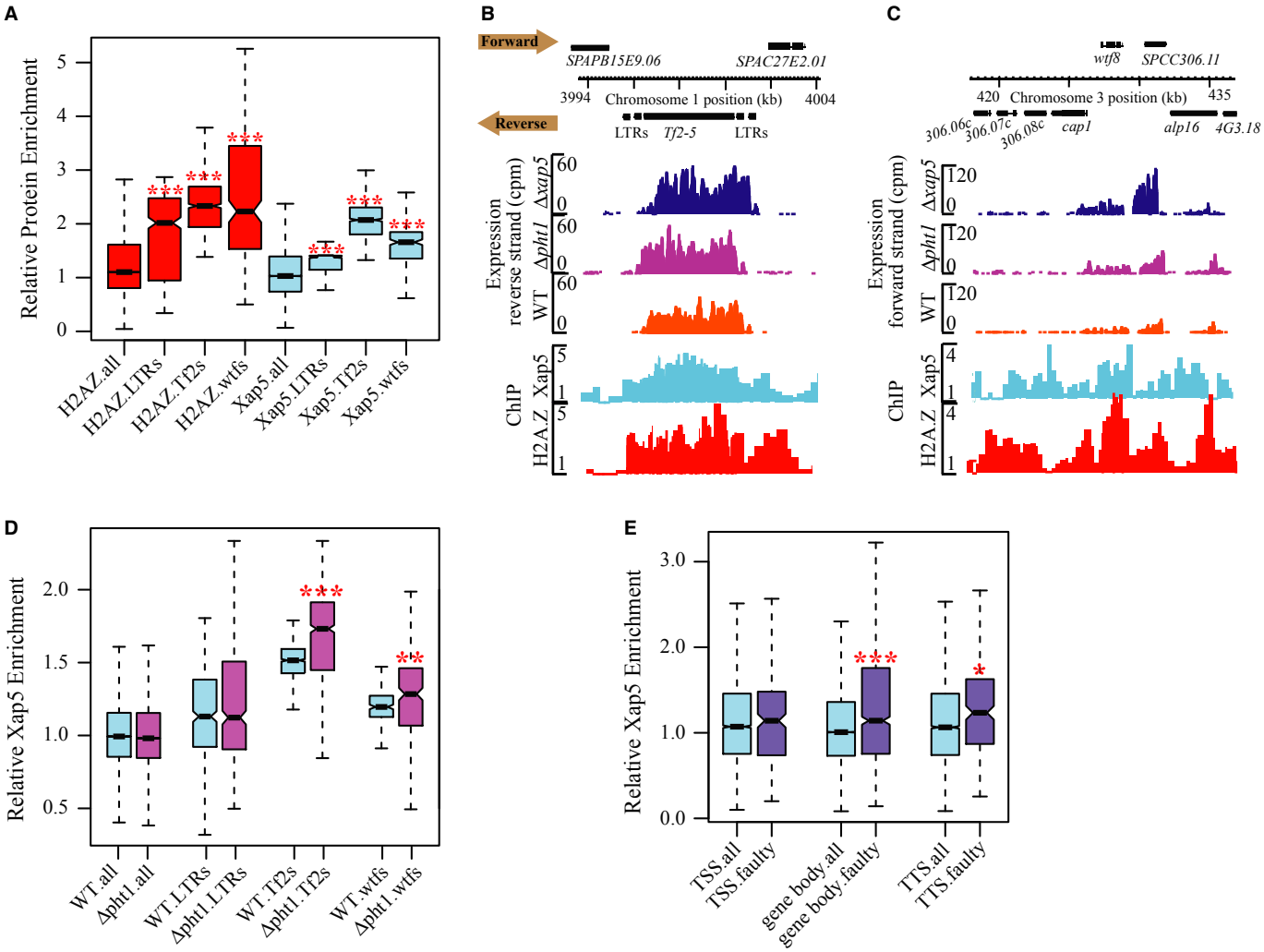


Figure 4. Xap5 and Pht1/H2A.Z protein association with chromatin is enriched at repeat element loci.

A Association of both Pht1/H2A.Z and Xap5 proteins are significantly enriched at LTR, transposable element (*Tf2*s) and *wtf* loci compared to the overall genome deposition levels (H2A.Z.all and Xap5.all).

B, C Transposable element (B) and *wtf* loci (C) are enriched for association of both Pht1/H2AZ and Xap5 proteins. Sense transcription from reverse and forward strands is shown in (B) and (C), respectively. Sense transcripts are upregulated in both mutants at these and adjacent loci (see also Supplementary Table S5).

D Association of Xap5 protein is increased at *Tf2* and *wtf* loci in Δ*pht1* relative to wild type.

E Association of Xap5 protein is significantly enriched in gene bodies and transcription termination sites (TTS) but not transcription start sites (TSS) of protein-coding loci with defective transcription termination in the Δ*xap5* mutant relative to all the genes in the genome.

Data information: In (A), (D), and (E): Welch's two-sample *t*-test (*P = 0.004, **P = 2.2e-4, and ***$P \leq$ 1.2e-15). Data are from one experiment; the same trend was observed in two independent biological replicates.

regulatory Xap5 proteins therefore awaits further, likely biochemical, studies.

Materials and Methods

Additional details are included in Supplementary Methods.

Growth analysis and gene transformations in *S. pombe*

Strains used are listed in Supplementary Methods. All yeast media and standard methods were according to Forsburg and Rhind [30].

Genetic interaction analysis

The epistatic miniarray profile (E-MAP) method was used [8,31]. Cells were grown at 20, 30 or 37°C in YES media.

RNA-seq, RT–PCR, and ChIP-chip analyses

Details and all primers used are listed in Supplementary Methods.

Data access

All raw data files are available at the NCBI Gene Expression Omnibus (GEO) repository under the accession number GSE46506.

Supplementary information for this article is available online: http://embor.embopress.org

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Author contributions

SA and SLH designed research; SA, AR, and MZ performed research; SA, AR, MZ, NJK, and SISG contributed new reagents/analytic tools; SA, AR, MZ, and SLH analyzed data; and SA and SLH wrote the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

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