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

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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 survival 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), Axap5 grows slightly more slowly than wild type at 30°C and this is exacerbated under nonoptimal growth temperatures (Fig 1A and B). The growth kinetics of wild-type, xap5-xap5 (SPCC1020.12c), and xap5-xap5 (XCT) are virtually identical under nonoptimal growth conditions (Fig 1C). The growth of xap5-xap5 (XCT) mutant is rescued by expression of xCT (xap5-xap5 + xCT) (Fig 1D) or xap5-xap5 (XCT) transformed with either plant XCT (Axap5-XCT) or S. pombe xap5 (Δxap5-xap5) grown at 21°C, are virtually

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indistinguishable (Fig 1A and B). Indeed, the generation time is almost twofold longer for the Δxap5 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 Δxap5 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, \(P < 2.2 \times 10^{-16}\)) 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 Δxap5 or that have similar genetic interaction profiles. Mutants that positively interact with Δxap5 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 Δxap5 (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 Δxap5 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 Δxap5, we compared genome-wide gene expression profiles of yeast with deletions in either xap5 or *pht1*, as described in more detail below. The *pht1* and Δxap5 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 Δxap5 and selected chromatin mutants with positively correlated genetic interactions (Supplementary Table S2). Like Δxap5 mutants, Δpht1 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 Δxap5 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 Δxap5 mutants. As previously reported for Δpht1 [16], we found that hundreds of loci corresponding to multiple types of transcripts are misregulated in both Δxap5 and Δpht1 (Supplementary Dataset S2; Fig 2A) and that the transcriptomes of Δxap5 and Δpht1 mutants are positively correlated (Fig 1F). The Arabidopsis *XCT* gene almost completely rescues the misregulation of gene expression in Δxap5 (Fig 2A and B), consistent with its ability to rescue the Δxap5 growth phenotype (Fig 1A and B). The Δxap5 Δpht1 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 Δxap5 and Δpht1 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 \(\geq 2\)) in Δxap5, 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 Δxap5 and Δpht1 mutants (Fig 1F), many loci upregulated in Δxap5 are also upregulated in Δpht1 and Δxap5 Δpht1 (Fig 2C–H and Supplementary Fig S4A–H). Centromeres, telomeres and silent mating-type loci are not expressed in either the Δxap5 or the Δpht1 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 Δxap5 and Δpht1 and are synergistically overexpressed in Δxap5 Δpht1 (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 Δxap5 is not primarily due to misregulation of antisense transcripts (Supplementary Fig S4). Many (54%) of the antisense transcripts in Δxap5 and more than 90% of the antisense transcripts in Δpht1 [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
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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 S4A and S4B), 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 Δxap5Δpht1 double mutants display an enhanced increase in antisense transcripts (Fig 2B) very similar to that seen when the Δpht1 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 Δxap5, Δpht1, and Δxap5Δpht1 (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 ≤ 0.05) upregulated in Δxap5 and Δpht1, 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 ≤ 0.01) upregulated in the Δxap5 and Δxap5Δpht1 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 Δxap5 and Δpht1 mutants (Fisher’s exact test, P-values for LTRs = 1.203e-05 and wtf s < 2.2e-16 in Δxap5 and LTRs < 2.2e-16 and wtf s = 0.006443 in Δpht1). 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 Δxap5 and Δpht1 mutants (Figs 3E and 4F 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 Δxap5 mutants and no global change in Xap5 association with chromatin in Δpht1 mutants (Fig 4D and Supplementary Dataset S3). However, we found an appreciable increase in Xap5 association with Tf2 and wtf loci in Δpht1 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 Tf2s, 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 Δpht1 has previously been shown to synergistically increase expression of antisense RNAs in fission yeast [16]. We therefore compared the Δxap5 transcriptome with previously published microarray data for several gene-silencing mutants (clr6.1, clr4.681, and clr6.1Δclr3) and one RNAi mutant (Δdcr1) [4]. We found moderate but statistically significant levels of correlation between the transcriptomes of Δxap5 and all these mutants, with the highest correlation seen between Δxap5 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 Δxap5 and Δpht1 in five different histone deacetylase complex mutants including Δclr3 and Δclr6.1. Strand-specific RT–PCR revealed that these loci are upregulated in all of these mutants as well as in Δxap5 and Δpht1 (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 Δxap5. 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 Δxap5. 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 Δxap5 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 Δxap5 are upregulated, and among these 60% represent aberrant transcripts such as antisense and intergenic sequences while only 36% correspond to protein-coding gene...
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 3. Many repeat elements are significantly upregulated in the Δxap5 mutant.
A, B LTRs significantly upregulated (P ≤ 0.05) in the Δxap5 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 ≤ 0.01) in the Δxap5 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).
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.

References
transcription, nucleosome positioning, and histone modifications genome-wide in fission yeast. Mol Cell Biol 33: 4779 – 4792