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Antisense transcription is coupled to nucleosome occupancy in sense promoters

Zhiming Dai* and Xianhua Dai

Department of Electronics, School of Information Science and Technology, Sun Yat-Sen University, Guangzhou 510006, China

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ABSTRACT

Motivation: Genome-wide pervasive transcription is widespread in eukaryotes, revealing an extensive array of antisense transcription that involves hundreds of previously unknown non-coding RNAs. Individual cases have shown that antisense transcription influences sense transcription, however, genome-wide mechanisms of how antisense transcription regulates sense transcription remain to be elucidated.

Results: Here, we performed a systematic analysis of sense–antisense transcription and nucleosome occupancy in yeast. We found that antisense transcription is associated with nucleosome occupancy in sense promoters. Using RNA polymerase II inactivation data as a reasonable approximation to antisense transcription inactivation data, we further showed that antisense transcripts increase nucleosome occupancy in sense promoter regions they overlap, and reduce nucleosome occupancy in sense promoter regions around their transcription termination sites. These results reveal the previously unappreciated roles of antisense transcription in directing nucleosome occupancy in sense promoters. Our findings will have implications in understanding regulatory functions of antisense transcription.

Contact: zhimdai@gmail.com

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1 INTRODUCTION

Pervasive transcription is widespread in eukaryotic genomes (David *et al.*, 2006; Kapranov *et al.*, 2007; Katayama *et al.*, 2005). The pervasive transcription gives rise to many non-coding (nc) RNAs which are critical for biological processes such as transcriptional regulation and genome stability (Bernstein and Allis, 2005; Moazed, 2009). Some ncRNAs overlap protein-coding genes, leading to the interleaved organization of transcription. Among these overlapping ncRNAs are a class that are transcribed antisense to the protein-coding sense transcripts. Experiments on individual genes have shown that antisense transcripts repress sense transcription through transcriptional interference (Hongay *et al.*, 2006) and inhibitory histone modifications (Berretta *et al.*, 2008; Camblong *et al.*, 2007). How antisense transcripts regulate sense transcription across the genome has so far, however, not been elucidated.

*To whom correspondence should be addressed.

The nucleosome is the basic unit of eukaryotic chromatin. Nucleosome occupancy in promoter regions is important for regulation of gene expression (Mavrich *et al.*, 2008; Tirosh and Barkai, 2008; Yuan *et al.*, 2005). A natural question is whether antisense transcription regulates sense transcription by influencing nucleosome occupancy in sense promoters.

In this study, we analysed sense–antisense transcription and investigated into the mechanisms of how antisense transcripts regulate sense transcription on a genomic scale in yeast. We found that antisense transcription causes sense transcripts to show high nucleosome occupancy in promoter regions. We also found that dynamic change in nucleosome occupancy in sense promoters is coupled to change in antisense expression.

2 METHODS

Yeast open reading frame (ORF) transcript coordinate data [including transcription start site (TSS) and transcription termination site (TTS)] were taken from Xu *et al.* (2009). We only used transcripts with confidently mapped 5′ ends and 3′ ends for analyses, a total of 4912 ORF transcripts. Yeast ncRNA coordinate data (including TSS and TTS) were taken from van Dijk *et al.* (2011), a total of 1658 ncRNAs. We used the 4912 ORF transcripts and the 1658 ncRNAs to identify genome-wide sense–antisense pairs in *Saccharomyces cerevisiae*. If one ORF overlap one ncRNA and they are transcribed oppositely, this ORF–ncRNA pair is identified as sense–antisense pair. In this way, we identified 1163 sense–antisense pairs (Supplementary Table S1). We identified ORF transcripts whose promoters are overlapped by antisense ncRNAs, to analyse the regulatory effects of antisense transcription on nucleosome occupancy in sense promoters.

Genome-wide gene transcription activity (transcription rate) data were taken from Holstege *et al.* (1998). Genome-wide RNA polymerase (Pol) II occupancy data in both strands with nucleotide resolution were taken from Churchman and Weissman (2011). For each transcript (ORF and ncRNA), we calculated the average RNA Pol II occupancy across its transcribed strand. As genome-wide transcription activity data are not available for ncRNAs, we used the resulting value as a close approximation to transcriptional activity. For each promoter (600 bp upstream of the transcript in this study), the RNA Pol II occupancy profile is a vector of length 600, in which each element is the RNA Pol II occupancy value in each position. For each antisense-overlapped promoter, we calculated the Euclidean distance between its RNA Pol II occupancy profile and that of each promoter without antisense transcript.

Genome-wide nucleosome occupancy data in YPD medium were measured with 1-bp resolution by Kaplan *et al.* (2009), which were measured by deep sequencing. We also used another independent datasets of nucleosome occupancy *in vivo* (Lee *et al.*, 2007), which were measured by microarrays, to test the robustness to choice of datasets. For each promoter (600 bp upstream of the transcript in this study), each coding

region, and each interval of [−150, −1], [−300, −1], [−450, −1], [−300, −150], [−450, −300] and [−600, −450] in each promoter, we calculated normalized nucleosome occupancy in this region divided by its length. All data in this study were measured in YPD medium unless indicated.

Genome-wide nucleosome occupancy data before and after inactivation of RNA Pol II (*rbp1-1*, 2 h at 37°C) were taken from Weiner *et al.* (2010). Genome-wide nucleosome occupancy data in ethanol and galactose conditions were taken from Kaplan *et al.* (2009). Expression level data for sense transcripts and antisense transcripts in ethanol and galactose conditions were taken from Xu *et al.* (2009). We identified antisense transcripts that show significant down-regulation (<0.5-fold) in ethanol or galactose conditions relative to YPD medium, and sense transcripts that are significantly up-regulated (>2-fold) in ethanol or galactose conditions relative to YPD medium.

3 RESULTS

3.1 Antisense transcription is correlated with high nucleosome occupancy in sense promoters

We analysed nucleosome occupancy in sense promoters overlapped by antisense transcripts. We found that gene promoters overlapped by antisense transcripts show significantly higher nucleosome occupancy (Kaplan *et al.*, 2009) within 150 bp upstream of their TSSs than the other promoters ($P<10^{-18}$, Mann–Whitney U-test, Fig. 1A). Similar result could be reproduced when using another dataset of genome-wide nucleosome occupancy (Lee *et al.*, 2007) ($P<10^{-40}$, Mann–Whitney U-test, Supplementary Fig. S1), suggesting that this result is robust to the choice of datasets. We also found that ncRNA promoters overlapped by ORF transcripts show significantly higher nucleosome occupancy (Kaplan *et al.*, 2009) within 150 bp upstream of their TSSs than the other ncRNAs (−0.29 versus −0.59; $P<10^{-6}$, Mann–Whitney U-test). Moreover, ORFs whose promoters are overlapped by antisense transcripts show lower transcriptional activities (Churchman and Weissman, 2011) than the other ORFs (1.68 versus 2.08; $P<10^{-4}$, Mann–Whitney U-test), and ncRNAs whose promoters are overlapped by ORF transcripts also show lower transcriptional activities (Churchman and Weissman, 2011) than the other ncRNAs (0.95 versus 1.14; $P<10^{-10}$, Mann–Whitney U-test). These results suggest that the presence of antisense (sense) transcripts is associated with high nucleosome occupancy in their overlapped sense (antisense) promoters and low sense (antisense) transcriptional activity, which is consistent with previous observation that antisense transcription generally represses sense transcription (Berretta *et al.*, 2008). We found that antisense transcriptional activity (Churchman and Weissman, 2011) is not correlated with nucleosome occupancy in sense promoters in YPD medium (Pearson correlation coefficient, $R=-0.003$, $P=0.95$). Nucleosome occupancy is known to be determined by multiple factors, including DNA sequence, chromatin remodelers and so on. These factors have been shown to explain most of nucleosome occupancy *in vivo* (Segal and Widom, 2009). Even if antisense transcription influences nucleosome occupancy in sense promoters, its contribution to nucleosome occupancy should be smaller than the other nucleosome occupancy determinants. In one cellular condition, antisense transcriptional activity is thus likely not to be correlated with nucleosome occupancy that is largely coordinated by the other determinants. Nucleosome occupancy

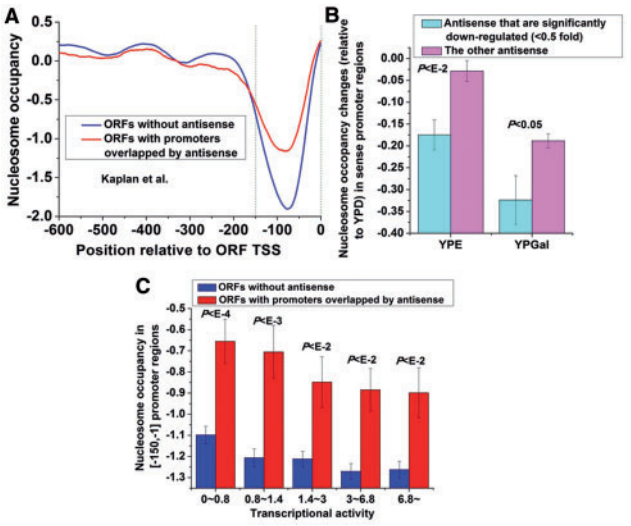

Fig. 1. Antisense transcription is correlated with high nucleosome occupancy in sense promoters. (A) Average nucleosome profiles in promoter regions are shown for ORFs with antisense-overlapped promoters and ORFs without antisense transcripts. (B) Average values that correspond to nucleosome occupancy changes (relative to YPD) in sense promoters are shown for ORFs whose paired antisense transcripts are significantly down-regulated (<0.5-fold) and ORFs whose paired antisense transcripts are not, depicted for two conditions: ethanol (YPE) and galactose (YPGal). Sense promoters whose paired antisense transcripts are significantly down-regulated (<0.5-fold) in ethanol or galactose conditions, show significantly reduced nucleosome occupancy than the other sense promoters. (C) Average values that correspond to nucleosome occupancy within 150 bp upstream of TSS are shown for ORFs with antisense-overlapped promoters and ORFs without antisense transcripts, depicted for five levels of ORF transcriptional activity. The result demonstrates that the correspondence between antisense transcription and high nucleosome occupancy within 150 bp upstream of sense TSS could be reproduced when controlling for sense transcriptional activities. Error bars in (B) and (C) were calculated by bootstrapping. The statistical significant values calculated from Mann–Whitney U-test are indicated in (B) and (C)

undergoes small changes among cellular conditions. These changes in individual promoters might be caused by changes in fewer nucleosome occupancy determinants, increasing the contribution of antisense transcription. Analysis of the dynamic data among cellular conditions might reveal the correlation between antisense transcriptional activity and nucleosome occupancy. We found that antisense transcriptional activity changes show significant positive correlation with nucleosome occupancy changes in sense promoters from YPDmedium to ethanol (galactose) condition (Pearson correlation coefficient, $R=0.19$, $P<0.01$, for ethanol condition; $R=0.15$, $P<0.01$, for galactose condition; Fig. 1B, and Supplementary Figs S2 and S3). It is the difference in antisense transcription that correlates more strongly with difference in nucleosome occupancy.

However, the correlation between antisense transcription and high nucleosome occupancy in sense promoters may not be direct but an artifact caused by low transcriptional activities of sense transcripts, because genes with high nucleosome occupancy within 150 bp upstream of their TSSs have low transcriptional

activities (Tirosh and Barkai, 2008), and genes with antisense transcripts also have low transcriptional activities (see above). We sought to examine whether this correlation could be observed when controlling for sense transcriptional activities. To this end, we clustered genes into five groups according to their transcriptional activities (Holstege *et al*., 1998), and genes in the same group have similar transcriptional activities. We found that genes with antisense-overlapped promoters still show significantly higher nucleosome occupancy within 150 bp upstream of their TSSs than the other promoters when controlling for sense transcriptional activities (Fig. 1C and Supplementary Fig. S4). This result indicates that the correlation between antisense transcription and high nucleosome occupancy in sense promoters is not caused by sense transcriptional activities. Considering that GC content of DNA sequence may bias the sampling of nucleosome-associated reads (Chung *et al*., 2010), we controlled for GC content and similar results could be observed (Supplementary Fig. S5).

We next examined whether ncRNAs increase nucleosome occupancy in regions that they overlap. We identified genes whose promoter regions are overlapped by tandem ncRNAs. We found that these genes also show higher nucleosome occupancy in promoters than the other genes (<-0.19 versus -0.26; $P<0.05$, Mann–Whitney U-test). Genes with antisense transcripts also show higher nucleosome occupancy in coding regions than the other genes (0.17 versus 0.05; $P<10^{-13}$, Mann–Whitney U-test). We also identified 103 sense–antisense pairs between two ncRNAs. We found that ncRNAs with antisense ncRNAs show higher nucleosome occupancy within 150 bp upstream of their TSSs than the other ncRNAs (-0.37 versus -0.63), albeit with weak statistical significance ($P=0.09$, Mann–Whitney U-test). These results imply that increased nucleosome occupancy is a general feature for regions overlapped by ncRNAs.

3.2 Antisense transcription influences nucleosome occupancy in sense promoters

We further investigated into nucleosomal organization of sense promoters overlapped by antisense transcripts. As shown in Supplementary Figure S6, nucleosome occupancy around antisense TTSs is lower than that in antisense transcripts. We asked whether this nucleosomal feature of antisense transcripts influences nucleosome occupancy in antisense-overlapped sense promoters: sense promoter regions overlapped by antisense transcripts show high nucleosome occupancy and sense promoter regions around antisense TTSs show low nucleosome occupancy. As nucleosome occupancy is not uniform throughout ORF promoters (Fig. 1A) and antisense transcripts terminate at different regions in sense promoters, it might confound the results if we analyse nucleosome occupancy of all antisense transcripts together. We thus should analyse antisense transcripts according to their TTSs respect to sense transcript TSSs, respectively. We classified antisense-overlapped sense promoters into three clusters: those whose paired antisense transcripts terminate in [−300, −150] ($N=101$), [−450, −300] ($N=50$) and [−600, −450] ($N=29$) in sense promoters, respectively. For these three sense promoter clusters, [−150, −1], [−300, −1] and [−450, −1] are their respective regions overlapped by antisense transcripts, and [−300, −150], [−450, −300] and [−600, −450] are their respective regions around antisense TTSs. We found that the three sense promoter clusters show significantly higher nucleosome occupancy (Kaplan *et al*., 2009) in regions overlapped by antisense transcripts, and show significantly lower nucleosome occupancy in regions around antisense TTSs than the promoters without antisense transcripts (Fig. 2A, and Supplementary Figs S7 and S8). Similar result could be reproduced when using another dataset of genome-wide nucleosome occupancy (Weiner *et al*., 2010; Supplementary Fig. S9).

As nucleosome occupancy in gene promoter is correlated with gene transcription, we next examined whether the observation above is an artifact of sense transcription. If this is the case, the nucleosome occupancy characteristics of antisense-overlapped sense promoters in Figure 2A should disappear when controlling for gene transcription. We analysed this potential artifact in terms of gene transcriptional rates and RNA Pol II occupancy in gene promoters. First, using genome-wide transcriptional rate data (Holstege *et al*., 1998), for each gene with antisense-overlapped promoter, we calculated the difference value between its transcriptional rate and that of each gene without antisense transcript, and could identify the gene without antisense transcript whose transcriptional rate is the most similar to this gene. Compared to these identified genes with similar transcriptional rates to antisense-overlapped sense transcripts, we found that antisense-overlapped sense promoter show significantly higher nucleosome occupancy in regions overlapped by antisense transcripts, and show significantly lower nucleosome occupancy in regions around antisense TTSs (Supplementary Fig. S10A). Second, using genome-wide RNA Pol II occupancy data (Churchman and Weissman, 2011), for each antisense-overlapped promoter, we calculated the Euclidean distance between its RNA Pol II occupancy profile and that of each promoter without antisense transcript, and could identify the promoter without antisense transcript whose RNA Pol II occupancy profile is the most similar (i.e. the least Euclidean distance) to this promoter. Compared to these identified promoters with similar RNA Pol II occupancy profiles to antisense-overlapped promoters, we found that antisense-overlapped sense promoter show significantly higher nucleosome occupancy in regions overlapped by antisense transcripts, and show significantly lower nucleosome occupancy in regions around antisense TTSs (Supplementary Fig. S10B). Together, the nucleosome occupancy characteristics of antisense-overlapped sense promoters in Figure 2A could be observed when controlling for gene transcription. These results collectively demonstrate that the nucleosomal occupancy difference between antisense-overlapped sense promoters and the other promoters depicted in Figure 2A is not associated with sense transcription.

We finally tested whether antisense transcription influences nucleosome occupancy in sense promoters. The results above are based on correlation analyses, more analyses are thus needed to examine the causality. If the nucleosomal occupancy difference between antisense-overlapped sense promoters and the other promoters is caused by antisense transcription, this difference should disappear when antisense transcription is inactivated. As genome-wide nucleosome occupancy data are not available for inactivation of antisense transcription, we used

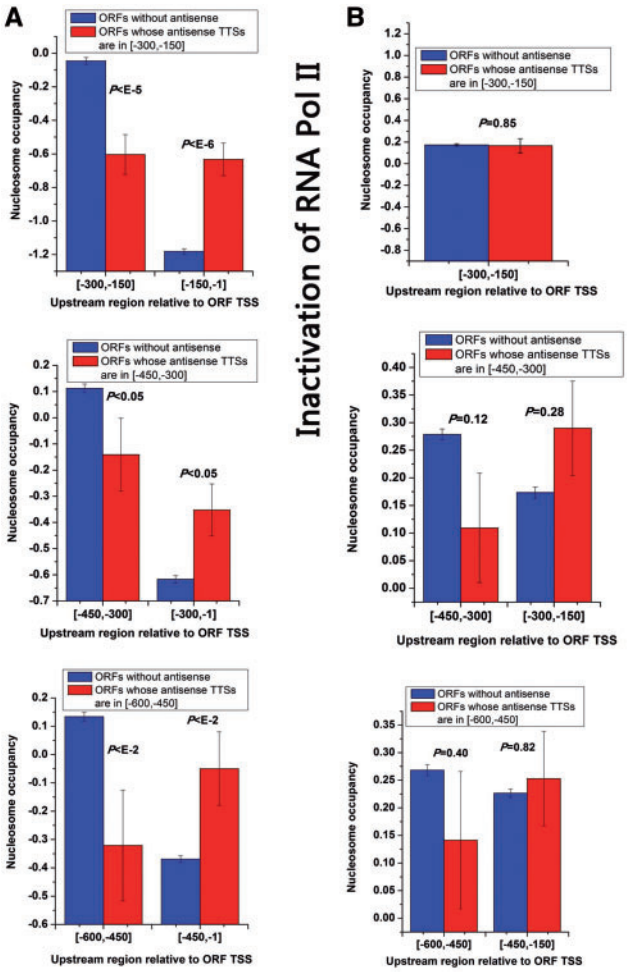

Fig. 2. Antisense transcription influences nucleosome occupancy in sense promoters. (A) Average values that correspond to nucleosome occupancy in antisense-overlapped regions and regions around antisense TTSs are shown for antisense-overlapped promoters, depicted for three types of sense promoters. For comparison, the corresponding regions are also shown for promoters without antisense transcripts. We excluded analysis to sense promoters whose paired antisense transcripts terminate in [−150, −1] because of their small overlap by antisense transcripts. The antisense-overlapped sense promoters show significantly higher nucleosome occupancy in regions overlapped by antisense transcripts, and show significantly lower nucleosome occupancy in regions around antisense TTSs. (B) Same as (A), but for nucleosome occupancy after inactivation of RNA Pol II (a close approximation to inactivation of antisense transcription, see details in main text). Considering that inactivation of RNA Pol II mainly influences nucleosome occupancy in nucleosome-free regions (i.e. [−150, −1] in gene promoters) and in coding regions, we excluded analysis to nucleosome occupancy in [−150, −1] in sense promoters, to avoid confounding the influence of antisense inactivation on nucleosome occupancy in this region, so the binning pattern in (B) is different from that in (A). The significant differences in nucleosomal occupancy between antisense-overlapped sense promoters and the other promoters depicted in (A) disappear in (B). The statistical significant values calculated from Mann–Whitney U-test are indicated in (A) and (B). Error bars were calculated by bootstrapping

genome-wide nucleosome occupancy data upon inactivation of RNA Pol II (Weiner *et al.*, 2010) as a close approximation. Although inactivation of RNA Pol II influences both antisense transcription and sense transcription, we have shown above that the nucleosomal occupancy difference between antisense-overlapped sense promoters and the other promoters is not associated with sense transcription, so the approximation above is reasonable in the analysis of the nucleosomal occupancy difference between antisense-overlapped sense promoters and the other promoters. Considering that inactivation of RNA Pol II influences nucleosome occupancy in nucleosome-free regions (i.e. [−150, −1] in gene promoters) and in coding regions (Weiner *et al.*, 2010), we excluded analysis to nucleosome occupancy in [−150, −1] in sense promoters, to avoid confounding the influence of antisense inactivation on nucleosome occupancy in this region. Upon inactivation of RNA Pol II (a close approximation to inactivation of antisense transcription), we found that the nucleosomal occupancy difference between antisense-overlapped sense promoters and the other promoters depicted in Figure 2A disappeared (Fig. 2B). This result shows that the nucleosomal occupancy characteristics in antisense-overlapped sense promoters are caused by antisense transcription. Taken together, these results demonstrate that antisense transcription influences nucleosome occupancy in sense promoters.

4 CONCLUSION

Individual cases have shown that antisense transcription influences sense transcription through transcriptional interference and histone modifications. However, genome-wide mechanisms of how antisense transcription regulates sense transcription remain to be determined. Here, we found that antisense transcripts are associated with high nucleosome occupancy in their overlapped sense promoter regions and low nucleosome occupancy in sense promoter regions around their TTSs (Supplementary Fig. S11). Moreover, dynamic antisense expression change is correlated with nucleosome occupancy change in sense promoters. These results reveal the mechanisms of how antisense transcription regulates sense transcription, and also provide new level of understanding the determinants of nucleosome occupancy in gene promoters. As we used RNA polymerase II inactivation data as an approximation to test the influence of antisense transcription on nucleosome occupancy, it will be very interesting to directly examine how antisense transcription regulates nucleosome occupancy in sense promoters by experiments.

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