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Title:

Extended ncRNAs interfere with promoter nucleosome dynamics

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Abstract:
Eukaryotic genomes generate vast numbers of ncRNA that can inhibit mRNA synthesis through transcription interference, but the mechanisms were unclear. A recent paper [1] shows that transcription of anti-sense, ncRNAs induces “elongation marks“ on histones in promoter regions. These inhibit active nucleosome positioning required to maintain open transcription-initiation sites.

Text: (1000 words, 10 references, 1 extra elements [figure, table, box])
A key feature of eukaryotic genomes is chromatin organization, which regulates gene expression, DNA replication and chromosome segregation. The basic unit of chromatin structure is the nucleosome, composed of eight histone proteins wrapped by two turns of DNA (~147 bp). The DNA in region upstream from the transcription start site (TSS) of active genes must be accessible to the transcription apparatus. To allow this, promoters are characterized by a region that is free of nucleosomes - nucleosome-depleted region (NDR, Fig. 1A); reviewed in [2]. Classical representations show NDRs with a sharp, uni-directional TSS. In fact, RNA polymerase (RNAP) II transcription from NDRs is inherently bidirectional. In some cases, this drives divergent gene expression, but more commonly the products are an mRNA plus an unstable, non-protein coding RNA (ncRNA) generated on the other strand [3]. The termination regions of protein coding genes are also permissive for the synthesis of antisense-oriented ncRNAs, possibly due to an open chromatin structure here. Moreover, transcription is subject to substantial stochastic noise, with low level RNAPII transcription detectable over almost the entire genome [4]. Together, these mechanisms generate very large numbers of ncRNAs. These ncRNAs are potentially damaging but are constantly cleared by the RNA surveillance machinery. Degradation is generally through 5’ or 3’ exonucleases, particularly the RNA exosome; reviewed in [5]. In yeast, the Nrd1/Nab3/Sen1 (NNS) complex recognizes short consensus motifs within RNA sequences and triggers both ncRNA transcription termination and exosome recruitment to degrade the product; reviewed in [6]. The recent work of Gill et al. [1] used sequestration of the NNS complex as a starting point to shed new light on mechanisms linking ncRNA synthesis to the maintenance of nucleosome organization.

Histone proteins frequently undergo post-translational modifications that are intimately connected with the regulation of chromatin organization and gene expression. Long, unstructured N-terminal regions, termed “tails”, on histone proteins can undergo methylation and/or acetylation at multiple sites. These modifications both reflect gene expression status and modulate transcription, acting either positively or negatively. Mapping data for

nucleosome positions generally represents an average, generated over many cells and potentially including nucleosomes in multiple states. “Textbook” figures resulting from such data can appear to indicate relatively static nucleosome locations and modification. In contrast, our current understanding of chromatin organization reveals it as a highly dynamic process.

Nucleosomes bind DNA with high affinity and some nucleotide preference, but this is insufficient to generate the *in vivo* chromatin structure [7]. Nucleosome positioning involves multiple chromatin remodeling factors, including the ATP-dependent, RSC complex (remodeling the structure of chromatin) [8]. In addition, the process of transcription strongly affects chromatin structure. The transcribing RNAPII carries a long, unstructured C-terminal domain (CTD) that can recruit histone modifying enzymes. These include Set2, which tri-methylates histone H3 on lysine 36 (H3K36me3) over the bodies of genes. Histones with the H3K36me3 modification are then recognized and bound by a histone deacetylases (HDAC) complex, called Rpd3S in yeast [9]. This results in removal of acetyl groups from histones H3 and H4, located elsewhere on the same nucleosome. Histone acetylation characterizes promoter regions of actively transcribed genes, particularly the -1/+1 nucleosomes flanking the NDR. Its removal within genes therefore reduces the chance of spurious RNAPII initiation, as well as polymerase collisions due to transcription from competing promoters (Fig. 1B).

A recent paper [1] proposes an elegant model linking antisense transcription with dynamic chromatin structure. The authors used an anchor-away system to restrict the NNS complex to cytoplasm, preventing its normal role in targeting antisense transcripts and promoting early termination. This revealed a subset of sense, protein coding genes, that were repressed upon depletion of NNS. The authors tracked the sequence of events leading to the repression: (i) NNS depletion impairs early termination of ncRNA, anti-sense transcripts, leading to greater elongation. (ii) Transcription elongation is accompanied by deposition of H3K36me3 along the body of the ncRNA transcription units. In the case of ncRNAs that lie antisense to protein coding genes, this can include the promoter of the sense transcript. (iii) H3K36me3 is a signal to remove H3 acetylation along the antisense transcript, including promoter of the sense, protein-coding gene. (IV) Deacetylation hinders recruitment of the RSC chromatin remodeling complex. This would normally bind the -1/+1, nucleosomes and maintain their separation, thus creating the NDR that is needed for promoter recognition. Reduced RSC association allows nucleosome sliding, which results in closure of the NDR and inhibits transcription initiation. Notably this implies that RSC normally acts dynamically to

hold the NRD in an open state. Altogether, these findings provide a mechanistic model for chromatin-driven transcription interference (Fig. 1C).

Subsequent bioinformatic analysis revealed that ~20% of yeast genes are subject to partial repression due to high levels of antisense transcription. The authors speculate that a defined, absolute level of antisense transcription must be attained to induce sense gene repression. These emerging mechanism indicate very dynamic regulation of TSS histone marks and extensive interplay between transcription units via chromatin organization (Fig 1D). It should be noted that other mechanisms likely remain to be found. Epigenetic systems are frequently characterized by functional redundancy, and yeast strains lacking Set2 are viable [10].

Interference between neighboring genes is a fascinating feature of gene transcription. A short-range mechanism, in which two molecules of RNAP transcribing DNA in opposite directions potentially collide and terminate, will cause competition between genes located in proximity. More complex, longer range, interference potentially arises from phase separation, driven by high local concentrations of transcription factors and weak multivalent interactions. Highly transcribed genes associate to form nuclear speckles (inter-chromatin granules), whereas proximity to compartments of low RNAPII transcription, e.g. the nucleolus, quenches transcription. The work spotlighted here describes how chromatin organization can mediate gene interference between neighboring genes, using histone marks as messengers, to avoid colliding RNAPII particles.

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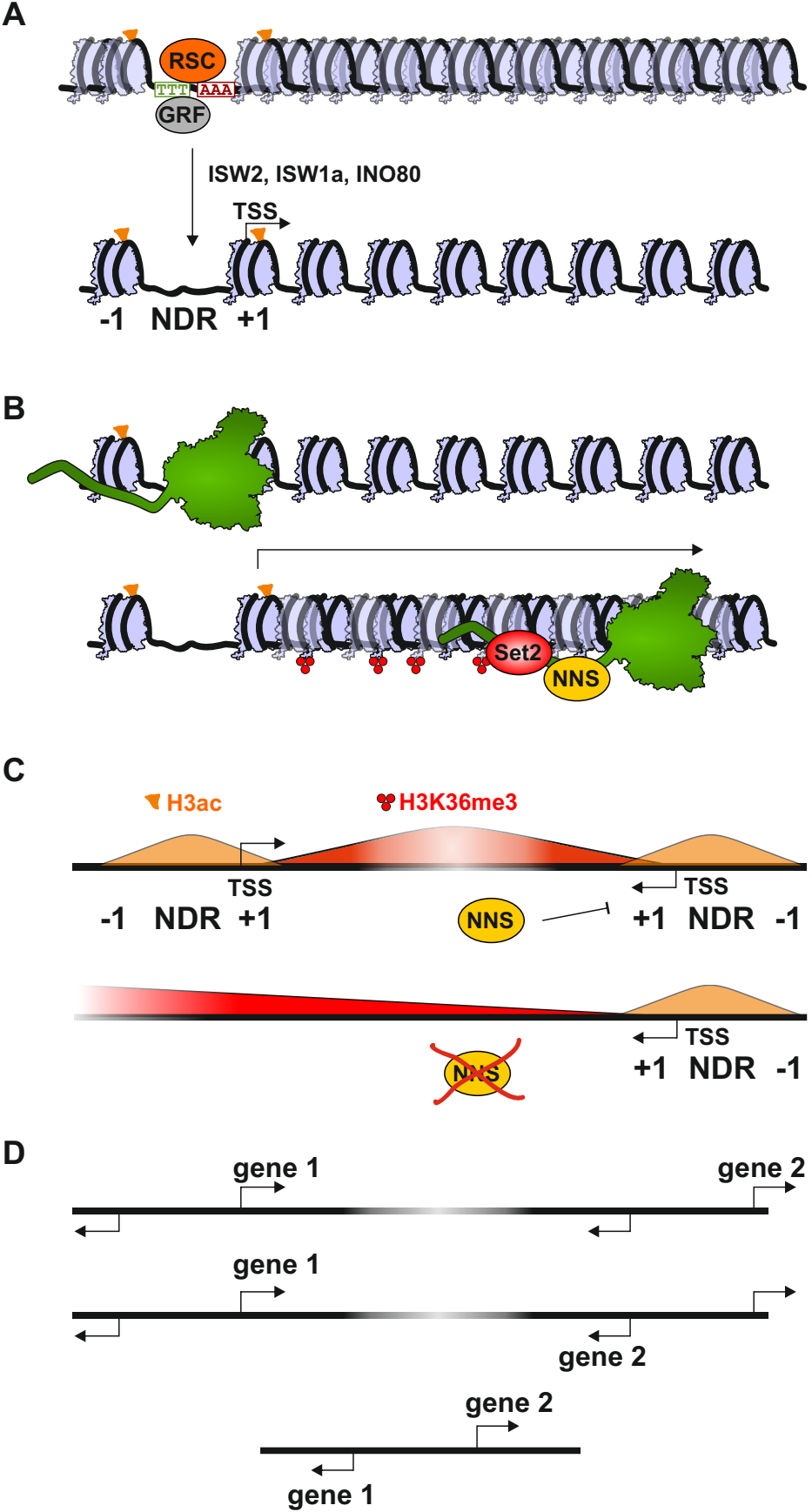
Figure legend:

A: Chromatin is remodeled by the RSC complex (regulation of chromatin structure) and general regulatory factors (GRFs), which create and maintain a nucleosome depleted region (NDR) at promoters [10]. ISW1a, ISW2 and INO80 set the +1 nucleosome position and align an array of nucleosomes [8].

B: RNAPII is a large, multiprotein complex with a molecular mass ~2 fold greater than nucleosomes, excluding the nascent RNA and associated proteins. DNA is wrapped twice around the nucleosome, establishing a tight structure that presents an obstacle to transcribing RNAPII. During transcription the DNA helix is also unwound as it passes through RNAPII, generating torsional stress and modulating nucleosome dynamics. The C-terminal domain of RNAPII associates with many factors, including the histone modifying enzyme Set2 and the NNS surveillance complex.

C: Model for transcription interference proposed by Gill et al [1], in which NNS depletion impairs early termination of antisense ncRNA transcripts. This leads to greater transcription elongation and consequent deposition of H3K36me3 along the extended transcription unit. The presence of H3K36me3 recruits an HDAC, which removes histone H3 acetylation from the nucleosome, potentially including those flanking the promoters of protein-coding genes. Deacetylation hinders recruitment of the RSC facilitating nucleosome sliding, and resulting in closure of the NDR, which inhibits transcription initiation.

D: Examples of interplay between transcription units. The dynamic regulation of TSS histone marks by competing transcription is expected to affect genes with multiple different relative organization. Unidirectional, divergent and convergent genes are all potentially susceptible to interference by sense or anti-sense transcription, either directly by flanking mRNA transcription, or the gene-associated ncRNAs. In all these cases deposition of H3K36me3 over the transcription unit could lead to closure of the NDR.


A
RSC
TTT
AAA
GRF
ISW2, ISW1a, INO80
TSS
-1 NDR +1
B
Set2
NNS
C
H3ac
H3K36me3
TSS
-1 NDR +1
NNS
TSS
+1 NDR -1
TSS
+1 NDR -1
D
gene 1
gene 2
gene 1
gene 2
gene 2
gene 1