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Transcription of ncDNA

Many roads lead to local gene regulation

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Transcription of ncDNA occurs throughout eukaryotic genomes, generating a wide array of ncRNAs. One large class of ncRNAs includes those transcribed over the promoter regions of nearby protein coding genes. Recent studies, primarily focusing on individual genes have uncovered multiple mechanisms by which promoter-associated transcriptional activity locally alters gene expression.

Genome-wide expression studies of multiple organisms, ranging from bacteria to humans, have revealed that transcription of non-coding DNA (ncDNA) accounts for a major portion of the transcriptional activity observed in cells. This activity not only yields a group of well-studied functional ncRNAs that include ribosomal RNAs, transfer RNAs, small nuclear RNAs and small nucleolar RNAs, but also an amazing array of previously uncharacterized ncRNAs that range in size from 18 nucleotides to many kilobases.[1-3] An emerging theme is that many of these ncRNAs play important roles in regulating gene expression. Trans regulatory mechanisms have been well documented including microRNAs, which inhibit translation or target mRNAs for degradation, and longer ncRNAs, such as mammalian *Xist*, *HOTAIR* and antisense *Kcnq1ot1* RNAs and Drosophila *roX* RNAs, which interact with protein complexes to modify chromatin structure.[1,3] At many other genomic locations, ncDNA transcription overlaps promoter and/or enhancer/silencer regions of protein-coding genes in either the sense or antisense direction. In this article, we highlight recent studies that elucidate a multitude of mechanisms by which transcription of ncDNA across gene regulatory sequences impact the expression of these genes.

Transcription of ncDNA Repositions Nucleosomes Over Promoter Regions

Chromatin dynamics at promoters play a key role during transcription regulation in eukaryotes. Many genes require the action of chromatin remodeling factors to remove or slide nucleosomes from their promoters to facilitate transcription factor binding and transcription initiation. Three recent studies have determined that transcription of ncDNA across gene promoters effectively alters the chromatin dynamics at these regions leading to dramatic changes in gene expression.[4-6]

In *Saccharomyces cerevisiae*, transcription initiation of *SER3*, a gene encoding an enzyme required for serine biosynthesis, is tightly controlled by the availability of serine. Initial studies of *SER3* regulation identified a ncRNA (named *SRG1*) that is expressed in serine-rich conditions from intergenic DNA, beginning 5' of the *SER3* gene and extending across the *SER3* promoter region.[7] Results from several experiments indicated that it is the act of transcribing *SRG1*, not the ncRNA product, which represses *SER3* by a transcription interference mechanism.[7,8] Subsequent studies showed that *SRG1* transcription regulates *SER3* expression by controlling nucleosome occupancy over the *SER3* promoter.[4] Nucleosome scanning and chromatin immunoprecipitation (ChIP) experiments indicated

Key words: ncDNA transcription, ncRNA, nucleosome, chromatin, histone modifications, transcription interference

Abbreviations: ncDNA, non-coding DNA; ncRNA, non-coding RNA; RNA Pol II, RNA Polymerase II; ChIP, chromatin immunoprecipitation; MNase, micrococcal nuclease; rDNA, ribosomal DNA

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that nucleosomes are randomly positioned over the *SER3* promoter and the presence of these nucleosomes is dependent on *SRG1* transcription. Analyses of mutant versions of the Spt6 and Spt16 histone chaperones, two factors that co-localize with elongating RNA Pol II and are required for transcription-dependent nucleosome assembly, provided strong evidence that nucleosomes assemble at the *SER3* promoter as a consequence of *SRG1* transcription and that these nucleosomes interfere with the binding of transcription factors at the *SER3* promoter. Based on these experiments, the following model has been proposed.[4] In a serine-rich environment, transcription of *SRG1* ncDNA is initiated upstream of the adjacent *SER3* gene and extends across the *SER3* promoter. During *SRG1* transcription, Spt6 and Spt16 reassemble nucleosomes over the *SER3* promoter after the passage of RNA Pol II, which then interfere with transcription factor binding resulting in *SER3* repression. In the absence of serine, *SRG1* transcription is down-regulated resulting in reduced nucleosome occupancy over the *SER3* promoter, which in turn allows activation of *SER3* transcription.

While *SER3* regulation involves a mechanism where transcription of intergenic ncDNA assembles nucleosomes over a promoter to silence adjacent gene expression, the reverse has also been observed. In *Schizosaccharomyces pombe*, transcription of the *fbp1*$^+$ gene is strongly induced in response to glucose starvation. A recent study detected several species of ncRNAs that are transiently expressed in response to glucose starvation prior to the production of *fbp1*$^+$ mRNA.[5] These transcripts initiate from several sites within the intergenic region 5' of *fbp1*$^+$ and extend across its promoter to the 3' end of this gene. MNase accessibility assays indicated a step-wise remodeling of chromatin—converting chromatin to a more open conformation—that parallels the appearance of the ncRNAs in response to glucose starvation. Blocking intergenic transcription by the insertion of a transcription terminator prevents this chromatin remodeling event and *fbp1*$^+$ activation is severely attenuated. In this case, transcription of intergenic ncDNA remodels promoter nucleosomes to increase the accessibility of the DNA to RNA Pol II and transcriptional activators.

Transcription of ncDNA has also been reported to facilitate induction of the chicken lysozyme gene.[6] However, in this case, transcription-dependent chromatin remodeling inhibits the binding of a repressor rather than permitting the binding of an activator. Transcription of the lysozyme gene is induced in response to lipopolysaccharide (LPS), which parallels changes in the DNase I hypersensitive sites within the regulatory regions upstream of the gene. MNase accessibility assays revealed that this change in DNase I hypersensitivity is the result of repositioned nucleosomes that then interfere with the binding of CTCF, a sequence-specific transcriptional repressor.[9,10] Using strand-specific RT-PCR, a non-coding transcript termed LINoCR, initiating 5' of the lysozyme gene promoter and extending across several cis-regulatory elements in an antisense direction, was detected in response to LPS induction.[6] ChIP and nucleosome scanning assays in the presence and absence of a transcription elongation inhibitor indicated that LINoCR transcription is responsible for repositioning the nucleosomes that inhibit CTCF binding leading to increased lysozyme gene expression.

Transcription of ncDNA Alters Post-Translational Modifications of Histones within Promoter Nucleosomes

Studies in both yeast and mammalian cells have recently shown that transcription of ncDNA at the promoters of protein-coding genes can regulate the expression of these genes by altering post-translational modifications of histones that occupy these promoters.[11-17] In some cases, the act of transcribing these regions brings about changes in histone modifications that reflect this activity, while other cases are dependent on the ncRNA product for recruitment of histone modifying enzymes.

In *S. cerevisiae*, two recent studies identified a ncRNA transcript (*GAL10* ncRNA or *GAL1ucut*) that initiates near the 3' end of *GAL10* and extends across the promoter region shared by the divergent *GAL1* and *GAL10* genes into the *GAL1* coding sequence.[11,12] Both groups provided evidence that transcription across the *GAL1-10* promoter, not the ncRNA product, attenuates expression of these genes using a mechanism similar to what has been described for the repression of aberrant transcription from cryptic promoters located within protein coding sequences.[18] Histone modifications, including histone H3 K4 and K36 methylation and H4 deacetylation, which are hallmarks of transcription activity, correlate with ncDNA transcription across the repressed *GAL1-10* locus. Mutations in the genes encoding subunits of the Rpd3S complex—the complex responsible for transcription-dependent deacetylation of histone H4—alter the expression of *GAL1* and *GAL10* to a degree similar to that observed in the absence of ncDNA transcription. Taken together, these data indicate that transcription of ncDNA across the *GAL1-10* promoter directs a cascade of histone modifications thus creating a chromatin environment that inhibits the binding of transcription factors.

Several studies involving human cells have shown that ncRNA products of antisense transcription can also silence nearby human genes by directing histone modifications to the promoter regions of these genes.[15-17] In one study, a 34.8 kb ncRNA transcript was found to be transcribed antisense to *p15*, a human tumor suppressor gene that encodes a cyclin-dependent kinase inhibitor implicated in leukemia.[17] This antisense transcript, termed *p15AS*, initiates 3' of the *p15* gene and extends beyond the promoter region of this gene. Expression studies involving several leukemia cell lines indicated an inverse correlation between *p15* and *p15AS*, suggesting that *p15AS* may be involved in silencing *p15* expression. Analysis of a series of *p15*/*p15AS* reporter constructs confirmed *p15* silencing by *p15AS* and indicated that *p15AS* acts in trans. Subsequent ChIP experiments detected increased H3 K9 dimethylation and decreased H3 K4 dimethylation across the *p15* promoter region, suggesting that this antisense ncRNA directs changes to histone modifications that lead to the formation of heterochromatin and gene silencing. Similar trans regulatory roles for antisense ncDNA

transcription in directing local heterochromatin formation have been described for the silencing of human genes expressing the *p21* tumor suppressor gene and the Oct4 pluripotency factor.[15,16] Interestingly, RNA knockdown experiments that targeted the *Oct4* antisense transcript suggest that these antisense transcripts may be directly involved in recruiting the histone modifying enzymes that promote heterochromatin formation.[15]

A more recent study indicates that the recruitment of histone modifying enzymes to promote heterochromatin formation and gene silencing is not limited to antisense transcripts.[14] PRC2 is a multisubunit complex conserved across metazoa that methylates histone H3 K27, a hallmark of repressed chromatin in mammals.[19,20] Investigation of PRC2-repressed genes in multiple human cell lines using genome-wide microarray analysis resulted in the detection of short ncRNAs (50–200 nucleotides) that are transcribed from regions flanking the transcriptional start sites of these genes.[14] Based on in vitro binding and immunoprecipitation assays, these ncRNAs were found to contain stem-loop structures that are recognized by the Suz12 subunit of PRC2 and this interaction is required to recruit PRC2 to its target gene. Once at these genes, PRC2 methylates histone H3 K27 within nearby nucleosomes, leading to heterochromatin formation and transcriptional silencing. Interestingly, PRC2 has previously been shown to interact with long ncRNA transcripts, such as with *Xist*, *HOTAIR* and *Kcnq1ot1*, to facilitate repression of their more long-range targets in trans.[20]

Repression in response to DNA damage of the human *CCND1* gene, which encodes a cell cycle regulator, has also been linked to changes in histone modifications caused by promoter-associated transcription.[13] Multiple ncRNAs have been detected initiating from intergenic sequences 5' of the *CCND1* promoter and extending bidirectionally.[21] However, the ncRNA products in this case indirectly affect histone modifications by inhibiting the binding of a sequence-specific transcription factor. An RNA binding protein, TLS, is recruited to the *CCND1* promoter through a direct interaction with the ncRNAs transcribed from this region, where it inhibits the binding of the CREB transcriptional activator.[13] This, in turn, prevents the recruitment of the p300 histone acetyltransferase, leading to reduced acetylation of histone H3 K9 and K14 and *CCND1* repression.

A Promoter-Transcribed ncRNA Alters Both Histone Modifications and DNA Methylation to Silence rDNA

In mammals, the silencing of ribosomal DNA (rDNA) by heterochromatin formation is mediated by small ncRNAs that are transcribed from rDNA promoter regions.[22] These ncRNAs (termed pRNAs for promoter-associated RNAs) are transcribed by RNA Polymerase I—initiating within the intergenic spacer regions between rDNA repeats and extending across the rDNA promoter—and then degraded or processed into stable 150- to 250-nucleotide pRNAs. These transcripts play two distinct roles in rDNA silencing: an indirect role in recruiting histone modifying enzymes and a direct role in recruiting DNA methyltransferases. First, pRNAs are responsible for the recruitment of the chromatin remodeling NoRC complex, a key regulator of mammalian rDNA silencing responsible for the subsequent recruitment of histone-modifying enzymes that mediate heterochromatin formation.[23,24] Recruitment of NoRC requires a conserved stem-loop secondary structure within pRNAs that is recognized by the Tip5 subunit of NoRC.[25] However, more recent studies have shown that although the stem-loop structure of pRNAs is necessary and sufficient for NoRC recruitment and heterochromatic histone modifications, additional pRNA sequence is required for de novo DNA methylation of this region and complete transcriptional silencing of rDNA.[22] Mapping studies identified a small region of pRNA that is required to recruit a DNA methyltransferase, DNMT3b, to the rDNA promoter and showed that this region overlaps the binding site for the TTF-1 transcription factor. Interestingly, in vitro binding studies determined that this region of pRNA and its matching DNA sequence form an RNA-DNA triplex structure that is recognized directly by DNMT3b. Taken together, these studies identify two regions of pRNA that form distinct structures recognized by rDNA silencing factors: (1) a stem-loop structure that recruits a histone modifier (NCoR) and; (2) a DNA-RNA triplex that recruits a DNA histone methyltransferase (DNMT3b).

A Promoter-Transcribed ncRNA Can Directly Interfere with Pre-Initiation Complex Formation

Several well-studied ncRNAs, including *6S* RNA, *7SK*, *B2* RNA and *Alu*, regulate transcription of distantly located genes by interacting with RNA Pol II or its associated factors.[26] A recent study indicated that this type of trans regulatory mechanism is also employed locally by a ncRNA transcribed at the promoter of its target gene.[27] Studies examining the repression of the human dihydrofolate reductase gene (*DHFR*) in quiescent human cells detected a ncRNA transcript expressed from an alternative promoter located 5' of the primary *DHFR* promoter.[28,29] Transcription from this alternative promoter represses *DHFR* expression by interfering with the assembly of the pre-initiation complex including RNA Pol II and its associated factors.[29] In this case, repression occurs in trans, requiring the region of the ncRNA transcript that overlaps the primary *DHFR* promoter.[27] Binding assays, both in vitro and in vivo, indicated that this ncRNA forms a ribonucleoprotein complex with TFIIB that prevents further recruitment of RNA Pol II and its associated factors. Interestingly, this ncRNA has also been reported to form an RNA-DNA triplex with its matching *DHFR* promoter DNA that may further contribute to *DHFR* repression by interfering with the binding of Sp1 transcription factors.

Concluding Remarks

Since the discovery that transcription is not limited to protein-coding genes and a few classes of functional RNAs, but occurs across entire genomes, there has been much debate concerning the functional significance of this activity. In some cases, ncDNA transcription may

simply be a by-product of either the pervasiveness of RNA polymerases or the normal regulation of a protein-coding gene.[30-34] However, considerable efforts of many laboratories have clearly demonstrated that transcription of ncDNA greatly impacts gene expression. Although ncRNAs often regulate target genes that are at a distance from their site of expression, many ncRNAs are transcribed from locations that include the promoter of nearby genes. For the genes that have been studied thus far, promoter-associated transcription often influences their expression. This report highlights several paths by which transcription of ncDNA across nearby gene promoters can achieve a common regulatory goal, which is to alter the binding of transcription factors to either activate or repress their target genes. Many unanswered questions remain regarding the generality and conservation of these mechanisms, necessitating the identification and characterization of more cases of promoter-associated transcription of ncDNA. Ultimately, it will be interesting to determine if specific genomic and/or molecular characteristics of promoter-associated transcription define the mechanism by which it may regulate gene expression.

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