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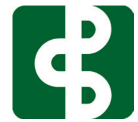

Short review

Gene regulation via long non-coding RNAs – lessons from yeast

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Abstract

Long non-coding RNAs have in recent years emerged as regulatory molecules in their own right impacting transcriptional regulation at the level of chromatin. Long non-coding RNAs have also been implicated in regulation of embryogenesis and tumor initiation, progression and metastasis. Regulation of gene transcription in yeast underpins a diverse array of cellular processes including metabolic regulation, sporulation and growth responses to nutrient deprivation. For most of these cases the transcription factors that regulate these processes have served as paradigms for our understanding of gene regulation in yeast and mammalian cells. More recently, an additional layer of transcriptional control in yeast has been uncovered in the form of long non-coding RNAs which originate as anti-sense transcripts of known genes or as intergenic transcripts overlapping gene promoters. These long non-coding RNAs and their transcription through promoter regions exhibits complex effects that directly affect promoter conformation at the level of histone modifications and chromatin structure. In this review we summarize some of the best characterized examples of transcriptional control through long non-coding RNAs and suggest that studies in yeast will greatly inform our understanding of the mechanisms of action of long non-coding RNAs in human cells.

INTRODUCTION

Transcriptional regulation lies at the core of how gene expression is regulated in response to external stimuli and endogenous signals for differentiation and development. Over the last three decades we have come to appreciate the complexity of transcriptional regulation through the action of a multitude of protein activators and repressors, coactivators and corepressors and general transcription factors (*1, 2, 3*). Additionally, regulation at the level of chromatin via histone modifications and nucleosome remodeling has been shown to be no less complex (*4, 5, 6*). More recently, we have become witness to the fact that non-coding RNA is itself an active player in transcriptional regulation. In particular, long non-coding RNAs (lncRNA) arising either from anti-sense gene transcription or intergenic transcription and overlapping with gene specific promoters have been found to be more than just transcriptional noise and play specific roles in modulating adjacent gene transcription directly.

By definition long non-coding RNAs lack an open reading frame and are longer than 200 nucleotides in length *(7)*. The ENCODE project has

identified more than 9000 human lncRNA loci of which approximately 40% overlap protein coding genes *(8, 9).* Long non-coding RNAs have been shown to bind DNA, RNA and/or proteins resulting in the potential for a variety of regulatory roles. However, despite their identification as lncRNAs very few have been functionally defined as true regulatory molecules. Similar to mRNAs lncRNAs can be modified via 5' capping, polyadenylation, splicing and RNA editing *(8, 9).* Multiple transcription factors have been associated with activation of lncRNA expression including Nanog, Sox2, Oct4, p53 and NFKB *(10).* As Nanog, Sox2 and Oct4 are master regulators of the stem cell phenotype it is intriguing to suggest that lncRNAs may also be implicated in regulating "stemness". While XIST RNA *(11)* and H19 *(12)* have served as paradigmns for lncRNA control of X-chromosome inactivation and imprinting respectively, a number of lncRNAs have emerged as regulators of tumorigenesis and metastasis. Perhaps the most studied of these is the lncRNA HOTAIR. HOTAIR was one of the first lncRNAs shown to regulate chromatin structure *in trans*. HOTAIR is overexpressed in approximately 25% of breast cancer patients *(10)* as well as being overexpressed in colon, liver and pancreatic cancers *(13–16).* In breast cancer, HOTAIR overexpression leads to breast cancer metastasis as shown in in vivo assays *(17).* HOTAIR is transcribed from the human HOXC locus on chromosome 12, interacts with the catalytic subunit of the polycomb repressive complex PRC2 and the H3K4 histone demethylase LSD1, thus signifying a direct link between lncRNA and chromatin modification *(7).* Significantly, HOTAIR operates in *trans* to regulate gene expression not at its native locus but rather at the distinct HOXD locus on chromosome 2 *(18).*

The bakers yeast *Saccharomyces cerevisiae* has long served as a model organism for many cellular processes that are conserved from yeast to man. As in human cells genome wide studies of the yeast transcriptome have uncovered an abundance of non-coding RNA transcripts the majority of which have yet to be functionally characterized *(19–22).* In this review we summarize some of the most studied examples of transcriptional regulation via lncRNA in the yeast Saccharomyces cerevisiae and glean principles of mechanism that may apply generally to regulation via lncRNAs in human cells as well.

Transcriptional paradigms meet lncRNA control

One of the first examples of ncRNA control of gene transcription in yeast was observed for the regulation of *SER3* transcription *(23, 24). SER3* is a gene required for serine biosynthesis and is regulated by a lncRNA, *SRG1*, which is transcribed through an intergenic region that overlaps with the *SER3* promoter. Transcription of *SRG1* acts to repress the downstream *SER3* gene. Under conditions when serine levels in the growth media are high, *SER3* RNA levels are low while *SRG1* levels are high. Conversely, when serine levels are low *SRG1* RNA levels are low and *SER3* RNA levels are high. Serine induction of *SRG1* ncRNA requires the transcriptional activator Cha4 (23) and the coactivator complexes SAGA and SWI/SNF *(23).* More detailed analyses showed that *SRG1* transcription leads to *SER3* inhibition by a mechanism in which *SRG1* transcription leads to an increased level of nucleosomes over the *SRG1* ncDNA which overlaps the *SER3* promoter *(24).* It has been suggested that transcription of the *SRG1* ncRNA leads to disassembly and reassembly of nucleosomes ahead of and behind the transcribing polymerase respectively. Nucleosome maintenance over the SRG1 ncDNA as evidenced by micrococcal nuclease protection experiments was shown to require the action of the transcription elongation factors Spt6 and Spt16 *(24).* Ultimately the nucleosome structure over the *SER3* promoter created by virtue of *SRG1* transcription likely results in defective binding of transcription factors required for *SER3* transcription.

The ability of yeast to metabolize galactose as an alternative carbon source is dependent on the activation of the *GAL* gene complement via the transcriptional activator Gal4 *(25). GAL* genes are normally repressed under conditions of high glucose, non-induced in raffinose and activated in the presence of galactose. A lnc anti-sense RNA originating from the 3'end of the *GAL10* gene, and subject to regulation by the Reb1 protein, was found to be transcribed under repressed (glucose) and non-induced conditions (raffinose) *(26).* Transcription of this lncRNA is associated with di- and tri-methylation of histone 3 lysine 4 residues within the *GAL10* gene and histone H3 lysine 36 tri-methylation across the entire *GAL1-10* region *(26, 27).* These repressive histone marks lead to decreased TATA box-binding protein and RNA polymerase II recruitment at the *GAL1* promoter and are eliminated in a *set1* histone methyltransferase mutant strain *(27).* Furthermore, deletion of the *Rpd3S* histone deacetylase complex subunit, *Eaf3*, which recognizes H3 lysine 36-trimethylation marks leads to derepression of the *GAL1-10* genes *(26).* Thus, active deactylation of histones contributes to the repressive effect of the lncRNA.

An additional nc RNA termed *GAL10s*, a sense oriented transcript originating upstream of the *GAL7* gene has been reported *(28).* In the absence of the RNA helicase *DBP2*, both *GAL10* and *GAL7* genes are more rapidly induced when cultures are shifted from the repressive (glucose) to the activated (galactose) condition. Furthermore, a defect in RNA decay (via xrn1 mutation) or RNA decapping (via dcp2 mutation) leads to accumulation of lnc-*GAL10* and nlc*GAL10s*. Surprisingly, this accumulation of lncRNAs correlated with rapid induction of *GAL1*, *GAL10* and *GAL7* mRNAs suggesting role for these lncRNAs in gene activation. In *dbp2* and *xrn1* mutant strains, RNA polymerase II is recruited to *GAL7* and *GAL10* promoters more rapidly suggesting a direct effect of lncRNAs on transcription initiation (28). Moreover, the

rapid induction of *GAL7* and *GAL10* in *xrn1* and *dbp2* mutant strains correlates with a lower occupancy of the transcriptional repressor Cyc8 at both promoter and 5' regions of the *GAL7* and *GAL10* open reading frames *(28)*. Notably, the *GAL* lncRNAs do not alter transcription induction from derepressed conditions but act only to kinetically enhance *GAL* gene induction from repressed conditions. This difference in growth conditions may account for the difference between the previously observed negative effect of lncRNAs and the subsequent observation that lncRNAs have a positive effect on *GAL* gene transcription. Given that the lncRNAs do not appear to effect a net increase in steady state levels of *GAL* gene transcripts but only affect induction kinetics it has been suggested that the lncRNAs act to poise *GAL* genes for rapid induction upon a shift from glucose to galactose media *(28)*.

The transcriptional induction of *PHO* genes underlies the response to phosphate availability in yeast *(29)*. It has been shown that under conditions of high phosphate availability *PHO* genes are repressed and induced when phosphate is low. In high phosphate conditions four positioned nucleosomes are found in the *PHO5* promoter region *(30)*. When phosphate is low, the transcriptional activator protein Pho4 translocates from the cytoplasm to the nucleus and binds to the *PHO5* promoter to activate transcription *(31)*. Activation involves eviction of the positioned nucleosomes by a number of factors including the chromatin remodelers SWI/SNF, INO80, RSC as well as SAGA and Asf1 *(32–37)*.

An intergenic anti-sense lncRNA was first observed at the *PHO5* promoter *(38)*. It was shown that transcription was not required for maintenance of histones at the promoter under repressive conditions but was required for normal kinetics of promoter remodeling under low phosphate conditions. Deletion of the 3' end of the *PHO5* open reading frame wherein the lncRNA begins showed that this leads to slower *PHO5* chromatin remodeling *(38)*. Thus, as described for the *GAL* locus, intergenic transcription affects the rate of activation and not the final steady state level of *PHO5* transcript.

In addition, two *PHO84* antisense transcripts were found to be stabilized in a *rrp6* mutant of the exosome *(39–42)*. Stabilization of the two antisense transcripts in this mutant background was found to be associated with repression of *PHO84* transcription. Interestingly, although loss of *RRP6* is associated with increased Hdac1 (histone deacetylase) recruitment to the *PHO84* as well as neighbouring genes, histone H3K18 deacetylation is restricted to the *PHO84* gene *(42)*. Given that this implicated the lncRNAs in repression via histone deacetylation, it was further shown that abrogation of lncRNA transcription prevents *PHO84* repression in the *rrp6* background. Unlike the other examples cited thus far in which lncRNAs act primarily in *cis*, the *PHO84* lncRNA is able to effect repression in *trans (41)*. However, subsequent single cell analyses of *PHO84* lncRNA localization showed that it is not stably associated with the *PHO84* gene in the nucleus but is rapidly exported to the cytoplasm *(39)*. This therefore suggests that the observation of *trans* repression may be through an indirect mechanism. Single cell analyses also showed that *PHO84* sense RNA and anti-sense lncRNA are strongly anti-correlated. *RRP6* was shown to favour early termination of the *PHO84* lncRNA via NNS thus indicating that stabilization in a *rrp6* background is the result of increased elongation of the lncRNA *(39)*. Indeed, depletion of the NNS subunit Nrd1 results in increased lncRNA. On the other hand deletion of the histone methyltransferase Set1 enhances the association of Nrd1 at the 3' end of the *PHO84* gene and reduces lncRNA transcription presumably through increased early transcription termination of the lncRNA *(39)*. Thus, Set1 and Rrp6 have antagonistic roles in the regulation of *PHO84* lncRNA transcription.

Nutrient deprivation among other things leads to haploid invasive growth and diploid filamentous growth both of which have been described as foraging responses *(43)*. Cell surface expression of the yeast *FLO11* gene is required for these alternate growth phenotypes. *FLO11* expression is variegated such that within a clonal population some cells express *FLO11* while others do not. This in turn leads to phenotypic heterogeneity and multiple growth phenotypes. Two *cis*-interfering lncRNAs designated *ICR1* and *PWR1* have been found to functionally impact the expression of *FLO11 (44, 45)*. *ICR1* initiates far upstream of the *FLO11* open reading frame and is transcribed across the promoter of *FLO11*, while *PWR1* lncRNA is transcribed from the *ICR1* complementary strand *(45)*. *ICR1* is associated with inhibition of *FLO11* transcription, and *PWR1* acts to activate *FLO11* transcription. Transcription of the two lncRNAs is regulated by the competitive binding of two transcription factors, Sfl1, which initiates *ICR1* transcription and Flo8 which initiates *PWR1* transcription *(45)*. Competition between the two lncRNAs determines whether the *FLO11* gene is active or repressed. Interestingly, Rpd3L a histone deacetylase was observed to be associated with activation of *FLO11*, even though it is normally required for repression at other promoters via chromatin condensation *(45)*. Analyses of various combinations of *rpd3L*, *flo8* and *sfl1* mutants, as well as Rpd3L binding to the *FLO11* promoter region, suggests that Rpd3L acts to condense chromatin in the *FLO11* upstream region causing inhibition of transcription of the *ICR1* negative lncRNA which then ultimately leads to *FLO11* gene activation. Single cell analyses using RNA FISH to examine *ICR1*, *PWR1* and *FLO11* transcripts verified a model in which alternative expression of lncRNAs contributes to variegated expression of *FLO11* in a clonal population *(44)*.

Sporulation of heterozygous yeast MATa/α diploid cells results in the formation of four haploid spores in response to nutrient deprivation. The transcriptional program re-

quired for sporulation has been shown to be primarily regulated by two genes, *IME1* (Inducer of Meiosis 1) *(46, 47)* and *RME1* (Repressor of Meiosis 1) *(48, 49).* In a or α haploid cells or homozygous diploid cells which cannot undergo sporulation *RME1* is the main repressor of *IME1*. However, in heterozygous diploid cells, *RME1* is not expressed and this allows for the induction of meiosis through *IME1* in response to nutrient deprivation. How *RME1* represses *IME1* in haploid cells has been an open question, and recent work has implicated a lncRNA termed *IRT1* (IME Regulatory Transcript) in repression of *IME1* in haploid cells *(50).* The *IRT1* transcript overlaps with the *IME1* promoter region and is expressed only in haploid cells or homozygous diploid cells. *IRT1* was directly implicated in *IME1* repression in experiments in which *IRT1* transcription was abolished via integration of a transcriptional terminator downstream of the *IRT1* transcription start site *(50).* In this case homozygous diploid and haploid yeast were found to express high levels of *IME1* in the absence of *IRT1*. Furthermore, binding of the transcriptional activator Pog1 to the *IME1* promoter was abrogated in the presence of the *IRT1* transcript *(38).* Subsequent analysis of nucleosome occupancy suggested that a repressive chromatin state over the *IME1* promoter is dependent on *IRT1* transcription and that this chromatin structure prevents recruitment of transcriptional activators to the *IME1* promoter *(50).* In addition it was shown that histone modifications associated with transcriptional repression and brought about by Set2 (histone methyltransferase) and Set3 (histone deacetylase) were increased in the *IME1* promoter region in haploid cells, further corroborating the existence of a repressive chromatin state on the *IME1* promoter.

A second ncRNA associated with regulation of sporulation is the *IME4* antisense transcript, *IME4-AS* (also known as *RME2*) *(51, 52).* The *IME4* gene encodes an RNA methyltransferase that, depending on the strain background, is either essential for initiation of sporulation or affects efficiency of the sporulation program *(53).* However, unlike the situation where *IRT1* acts to repression transcription initiation of the *IME4* gene, in this case the anti-sense transcript appears not to affect the promoter of the *IME4* gene but rather may play a role in regulating transcription elongation through the *IME4* open reading frame. This was suggested based on the observation that a 450 bp region internal to the *IME4* coding region is required in an orientation dependent manner for *RME2* dependent repression of *IME4 (51).* Additionally, it was observed that *RME2* can repress *IME4* expression when it is placed under the control of a heterologous *GAL1* promoter suggesting that the *IME4* promoter is not specifically associated with repression by the antisense RNA *(51).* A similar mechanism appears to be involved in the regulation of the meiosis specific gene *ZIP2*, wherein an antisense RNA (*RME3*) is required for haploid cell repression of *ZIP2 (51).* In both cases repression by the antisense transcripts occurs in *cis* but not in *trans (51, 52).*

CONCLUSION

It is clear from the examples described that lncRNAs in yeast play a crucial role in regulation of inducible gene expression in response to changes in extracellular conditions and we are likely to uncover more examples of lncRNA regulation in future. While there are subtle specificities to each case, a general mechanism emerging from these studies suggests that lncRNAs which overlap with gene promoters, lead to characteristic changes in gene expression by virtue of altering promoter chromatin and histone modifications and thereby accessibility to inducing transcription factors. Importantly, studies of the *PHO* genes and *GAL* genes in this regard suggest that kinetics of activation as opposed to steady state levels of activation are targeted by lncRNA regulation. It will be of considerable interest to see if similar types of regulation of critical transcription factors is the case in human cells as well. An important question that remains is the issue of whether lncRNA is primarily a phenomenon that acts in *cis* or in *trans.* From the examples in yeast and studies to date in human and mouse cells, the answer is likely that both types of regulation occur. This overview of lncRNA regulated gene expression in yeast supports a greater investment of scientific study in this burgeoning area and studies in yeast will surely yield mechanistic understanding of lncRNA function that apply to mammalian systems as well.

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