Roles of lncRNA transcription as a novel regulator of chromosomal function

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In recent years, many transcriptome analyses have revealed that numerous noncoding RNAs are transcribed in eukaryotic cells. Long noncoding RNAs (lncRNAs), which consist of over 200 nucleotides, are considered to be key players in a variety of biological processes and structures including gene expression, differentiation and nuclear architecture. Many studies on individual lncRNAs have identified their molecular functions as decoys, recruiters and scaffolds, which arise through interactions with proteins and the construction of ribonucleoproteins. In addition to the roles played by transcribed lncRNA molecules, several studies have indicated the important functions of nascent lncRNA transcription processes. In this review, we discuss recent findings on the important roles of lncRNA transcription processes in the regulation of chromosome function.

Key words: chromatin, histone modification, long noncoding RNA (lncRNA), RNA polymerase II (RNAPII), transcription

INTRODUCTION

Recent technical advances have enabled the high-throughput analysis of transcriptomes. Such transcriptome analyses have indicated that a large fraction of the eukaryotic genome, including intergenic and antisense regions, is transcribed into RNAs (Okazaki et al., 2002; Carninci et al., 2005; David et al., 2006; Dutrow et al., 2008; Nagalakshmi et al., 2008; Rhind et al., 2011). Indeed, while only 2% of the human genome comprises protein-coding regions, 70% of the genome is transcribed into RNAs (Djebali et al., 2012). Thus, the majority of RNAs apparently do not have protein-coding potential, and these RNAs are known as noncoding RNAs (ncRNAs). The ncRNAs are divided into two classes, namely small RNAs and long ncRNAs (lncRNAs), according to their length. Small RNAs are those ncRNAs comprised of less than 200 nucleotides; siRNAs and miRNAs are examples of small RNAs. Small RNAs regulate gene expression via degradation of mRNA and inhibition of translation through their interaction with RNA-induced silencing complexes (Carthew and Sontheimer, 2009). In contrast to small RNAs, lncRNAs are ncRNAs comprised of more than 200 nucleotides. They are involved in various biological processes, including gene regulation, development and nuclear organization (reviewed by Akkipeddi et al., 2020).

Studies of lncRNA function were initiated following the identification of XIST/Xist (Borsani et al., 1991; Brown et al., 1991) and roX (Amrein and Axel, 1997; Meller et al., 1997). These specific lncRNAs play a pivotal role in dosage compensation, an epigenetic process that equalizes gene expression from an unequal copy number of sex chromosomes. In Drosophila melanogaster, males with a single X-chromosome have the same level of X-linked gene expression as females with two X-chromosomes. This equalization is established by five proteins (MSL1, MSL2, MSL3, MOF and MLE) and two lncRNAs, namely roX1 and roX2 (Gu et al., 1998; Meller et al., 2000; Smith et al., 2000; Conrad and Akhtar, 2012). The male-specific lethal complex, consisting of these five proteins and the roX lncRNAs, coats the male X-chromosome and induces acetylation of histone H4K16 to enhance X-linked gene expression in a male-specific manner (Smith et al., 2000; Ilik et al., 2013). Contrary to this Drosophila compensation process, one X-chromosome in mammalian female cells is inactivated to generate equivalent levels of male X-chromosome genes. X-chromosome inactivation is established by Xist, which is a ~17-kb lncRNA transcribed from the X-chromosome. Xist coats one of the female X-chromosomes (Clemson et al., 1996; Simon et al., 2013) and recruits polycomb repressive complex 2 (PRC2) (Zhao et al., 2008), which methylates histone H3K27, leading to the formation of constitutive repressive heterochromatin. In addition to Xist, interactions between PRC2 and various lncRNAs, including HOTAIR and KcnqotI, have
been reported (Davidovich and Cech, 2015). These various studies reveal the pivotal roles played by lncRNAs in the regulation of gene expression through chromatin modification via recruitment of histone modification factors.

Some lncRNAs also act as part of the nuclear architecture. For example, MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), an abundant nuclear lncRNA carrying more than 8,000 nucleotides, is localized at nuclear speckles (Ji et al., 2003; Hutchinson et al., 2007), which are nuclear domains, also known as interchromatin granule clusters, associated with splicing factors (Spector and Lamond, 2011). In one study, MALAT1 was found to be non-essential for the formation of nuclear speckles and mouse survival (Nakagawa et al., 2012). In human cells, MALAT1 regulates alternative splicing by modulating phosphorylation of pre-mRNA splicing factors (Tripathi et al., 2010). In addition, upon serum stimulation, MALAT1 is involved in the regulation of growth control genes through the relocation of the genes’ loci from repressive polycomb bodies to interchromatin granules (Yang et al., 2011). NEAT1 (nuclear enriched abundant transcript 1) is another lncRNA localized in the nuclear architecture, specifically at paraspeckles, which are ribonucleoprotein complexes composed of several proteins and RNA. NEAT1 is essential for the formation and maintenance of paraspeckles (Clemson et al., 2009; Sasaki et al., 2009). Thus, these collected studies reveal the pivotal role of specific lncRNAs, namely NEAT1 and MALAT1, as a part of the nuclear architecture associated with transcriptionally active regions (West et al., 2014).

Many studies of lncRNAs have demonstrated the important roles they play in diverse biological processes through their interactions with various proteins including histone modification factors and nuclear architecture proteins. In addition to the roles played by transcribed lncRNA molecules, several studies have suggested the importance of cis-acting RNA polymerase II (RNAPII) that transcribes nascent lncRNAs in the regulation of neighboring genes through their effects on chromatin configuration or on the action of RNAPII. In this review, we highlight the roles of lncRNA transcription-mediated chromatin modulations in the gene regulation involved in metabolism, sexual differentiation and development in yeasts and mammalian cells.

**REGULATION OF SER3 BY INTERGENIC lncRNA (SRG1) TRANSCRIPTION IN SACCHAROMYCES CEREVISIAE**

The *S. cerevisiae* gene SER3 encodes a phosphoglycerate dehydrogenase that catalyzes serine biosynthesis (Albers et al., 2003) and it is repressed in serine-containing medium (Martens and Winston, 2002). Martens et al. (2004) discovered that RNAPII and TATA-binding protein bind to the region upstream of the SER3 TATA-box in serine-rich conditions. These upstream bound transcription apparatuses initiate transcription of the lncRNA SRG1 (SER3 regulatory gene 1) in serine-rich conditions, and this lncRNA transcription passes through the SER3 TATA-box (Fig. 1A). Mutation of the SRG1 TATA-box impairs SRG1 transcription and concurrently causes the derepression of SER3 expression. SRG1 expression in trans does not rescue the phenotype of the TATA-box mutant. Moreover, the repressive function of SRG1 transcription is preserved by the sequence replacement of a large region of SRG1. These observations indicate that SRG1 regulates SER3 transcription via a transcriptional interference mechanism (Martens et al., 2004). SER3 repression by SRG1 transcription is mediated by the binding of serine-responsive activator Cha4 to the SRG1 upstream activation site (UAS) through the facilitated recruitment of SAGA and SWI/SNF (Martens et al., 2005). Additional studies revealed that, for SER3 repression, SRG1 transcription is required for nucleosome assembly to the repressive chromatin configuration at the SER3 UAS (Hainer et al., 2011). Moreover, this SRG1 transcription-mediated nucleosome assembly is dependent on the Spt6 histone chaperone and Spt16 transcription elongation factors (i.e., the FACT complex). Taken together, these studies suggest the existence of a lncRNA transcription-mediated chromatin regulation, in which SRG1 transcription, but not the transcribed RNA molecule, is involved in the recruitment of Spt6 and Spt16 to assemble and maintain nucleosomes at the SER3 UAS for the repression of SER3.

**REGULATION OF IME1 THROUGH THE TRANSCRIPTION OF TWO lncRNAs (IRT1 AND IRT2) AT THE IME1 PROMOTER IN S. CEREVISIAE**

In yeast, transcription of lncRNAs also plays a critical role in the cell fate decision leading to gametogenesis, also known as meiosis and sporulation. This sexual differentiation takes place in diploid cells carrying two different mating types, MAT-a and MAT-a. To enter into gametogenesis, multiple signals including mating type are required so that the process does not occur in haploid cells. Diploid cells carrying both mating types (i.e., MAT-a and MAT-a) express both mating type-specific genes upon nutrient starvation, which leads to the formation of the a1/a2 repressor complex. This a1/a2 heterodimer plays key roles in the shift from vegetative growth to the onset of meiosis (reviewed by Honigberg and Purnapatre, 2003). IME1 (inducer of meiosis 1) encodes a transcription factor that serves as a master regulator for meiotic entry (reviewed by van Werven and Amon, 2011). In meiotic entry, the a1/a2 repressor complex represses expression of Rme1, an IME1 repressing factor, and thereby allows IME1 induction. However, several
Fig. 1. Gene regulation by lncRNA transcription in *Saccharomyces cerevisiae*. (A) In serine-rich medium, the serine-responsive activator Cha4 binds to the SRG1 upstream activation site (UAS) and recruits SAGA and SWI/SNF. SRG1 transcription passing through the SER3 promoter region induces a repressive chromatin structure by mediating Spt6 and Spt16 (the FACT complex). (B–E) Cell fate decision is mediated by long noncoding RNA (lncRNA). (B) In sporulation medium without two different mating-type signals (i.e., sporulation in haploid cells), IRT1 transcription is activated by the binding of Rme1 to two Rme1 binding sites. IRT1 transcription induces histone methylation (H3K36-me) by mediating Set2 histone methyltransferase. Set3 histone deacetylase binds to this modified histone and establishes repressive chromatin. (C) In sporulation medium with two different mating-type signals (i.e., sporulation in diploid cells), the α1/α2 repressor complex represses expression of Rme1, resulting in reduced Rme1 protein (small oval). Moreover, IRT2 transcription represses Rme1 binding and IRT1 transcription, and then Pog1 is permitted access to the IME1 promoter. The expressed Ime1 protein further activates IRT2 expression. This positive feedback circuit contributes to efficient IME1 expression and thereby enables cells to enter meiosis. (D) In haploid cells (during sporulation), RME2, an antisense lncRNA that includes the 5’ end of the IME4 ORF, is transcribed and thereby represses IME4 expression. (E) In diploid cells (during sporulation), the α1/α2 heterodimer represses RME2 transcription and IME4 is activated.
natural yeast species exhibit residual Rme1 expression during meiosis (Deutschbauer and Davis, 2005; Gerke et al., 2006), suggesting that another regulatory mechanism (or mechanisms) ensures meiosis-specific expression of IME1.

van Werven et al. (2012) first revealed that the transcription of two promoter-associated lncRNAs, namely IRT1 and IRT2 (IME1 regulatory transcript 1 and 2), is involved in the regulation of IME1 expression (Moretto et al., 2018). In sporulation medium, IME1 is transcribed in diploid MAT-a/α cells but not in haploid cells that possess only one type of the mating gene. In contrast, IRT1 is transcribed in haploid cells but not in diploid MAT-a/α cells. Thus, expression patterns of IME1 and IRT1 are mutually exclusive. The IRT1 transcript is a 1.4-kb lncRNA initiated from the region upstream of IME1 that overlaps the IME1 promoter. This lncRNA transcription recruits histone methyltransferase Set2, which induces the histone modification H3K36-me. IRT1 transcription also recruits a histone deacetylase, Set3, which binds to H3K4-m2 and thereby establishes a repressive chromatin state (Fig. 1B). IRT1 transcription is activated by Rme1 (Fig. 1B). This IRT1 transcription-mediated chromatin assembly prevents access of the transcription factor Pog1 to the IME1 promoter (van Werven et al., 2012) (Fig. 1B). On the other hand, IRT2 regulates IME1 expression positively. IRT2 is a 400-bp lncRNA initiated from a position adjacent to upstream of IRT1. IRT2 is expressed in the same direction as IRT1 and IME1 in haploid cells (Moretto et al., 2018). IRT2 transcription establishes a repressive chromatin structure at the IRT1 promoter and thereby inhibits the Rme1 binding required for IRT1 activation (Fig. 1C). Thus, IRT2 transcription activates IME1 indirectly through the repression of IRT1 transcription (Fig. 1C). Although IRT1-mediated IME1 repression depends entirely on Set2 histone methyltransferase, IRT2-mediated IRT1 repression does not (Moretto et al., 2018). In addition, Ime1 itself acts as an activator for IRT2. In summary, the cell fate decision leading to gametogenesis is determined by IME1 expression, which is strictly regulated by a regulatory circuit of two promoter-associated lncRNAs (IRT1 and IRT2) and Ime1.

REGULATION OF IME4 BY TRANSCRIPTION OF AN ANTISENSE lncRNA (RME2) IN S. CEREVISIAE

IME4 (inducer of meiosis 4), which encodes an RNA methyltransferase (Clancy et al., 2002), is one of the critical factors for the initiation of meiosis (Shah and Clancy, 1992). IME4 transcription is activated in MAT-a/α diploid cells, but not in MAT-a or MAT-α haploid cells, during meiosis (Shah and Clancy, 1992). In haploid cells, IME4 transcription is repressed by the transcription of an antisense lncRNA, RME2 (regulator of meiosis 2) (Fig. 1D) (Hongay et al., 2006; Gelfand et al., 2011). The a1/a2 heterodimer represses RME2 and thereby allows the induction of IME4 during meiosis in diploid cells (Fig. 1E) (Hongay et al., 2006; Gelfand et al., 2011). Given that RME2 expression in trans does not repress IME4 transcription, RME2 transcription-mediated IME4 repression is promoted in a cis-acting manner (Hongay et al., 2006). Together, these studies reveal that RME2 transcription, in the reverse direction to IME4 transcription and passing through the IME4 open reading frame (ORF), counteracts IME4 transcription. In the models of SRG1-1, IRT1-1 and IRT2-mediated transcriptional repression, lncRNA transcription passing through the promoter regions prevents transcription factor binding at the promoters. However, the binding of TATA-binding protein at the IME4 promoter is unaffected by RME2 transcription. Moreover, RME2 transcription passing through the 5’ end of the IME4 ORF is essential to the repression of IME4 expression; however, extension of the antisense transcript through the TATA-box of IME4 is not required for IME4 repression (Gelfand et al., 2011). These results suggest that RME2 transcription represses IME4 expression via a different mechanism to that underlying SRG1-SER3 regulation. A similar mechanism is observed for ZIP2, a gene that is regulated by an antisense lncRNA (RME3) in a cell type-specific manner (Gelfand et al., 2011).

REGULATION OF fbp1 BY STEPWISE TRANSCRIPTION OF lncRNAs (mlonRNAs) IN SCHIZOSACCHAROMYCES POMBE

The S. pombe gene fbp1 encodes fructose-1,6-bisphosphatase, which is required for gluconeogenesis, and it is transcriptionally regulated by environmental glucose concentration (Vassarottis and Friesens, 1985; Hoffman and Winston, 1989, 1990, 1991). The activation of fbp1 is mediated by two transcription factors, Atf1 and Rst2, binding at two upstream cis-acting elements, UAS1 and UAS2, respectively (Fig. 2A) (Neely and Hoffman, 2000; Janoo et al., 2001; Higuchi et al., 2002; Hoffman, 2005). Upon glucose starvation, several species of lncRNAs are transcribed from the region far upstream of the fbp1 promoter (Fig. 2A) (Hirota et al., 2008a). These lncRNAs are transcribed by RNAPII and are polyadenylated, which are both characteristics of mRNA. Therefore, these lncRNAs were initially defined as mRNA-type long ncRNAs (mlonRNAs) (Hirota and Ohta, 2009). However, subsequent to this definition, the abbreviation lncRNA has been used in this scientific field to represent “mRNA-type long ncRNA”; thus, the abbreviation mlonRNA was redefined as “metabolic stress-induced lncRNA” (Galipon et al., 2013). In glucose-rich medium, the longest mlonRNA (mlonRNA-a), which is initiated upstream of UAS1, is weakly transcribed (Fig. 2B). During glucose starva-
Fig. 2. Gene regulation by lncRNA transcription in *Schizosaccharomyces pombe*. (A) Schematic diagram of the *fbp1* upstream region containing upstream activation sites 1 and 2 (UAS1 and UAS2), which are binding sites for transcription factors Atf1 and Rst2, respectively. The metabolic stress-induced lncRNAs (mlonRNAs) initiating from the region upstream of the *fbp1* promoter and *fbp1* mRNA are shown. Numbers indicate the transcription start site of *fbp1* and mlonRNA transcripts from the first ATG of the *fbp1* open reading frame. (B) In glucose-rich conditions, positioned nucleosomes prevent transcription factor binding. Tup proteins, which are global corepressors, repress inappropriate chromatin remodeling and *fbp1* mRNA transcription. In glucose starvation conditions, Atf1 binds at UAS1 and induces the initiation of mlonRNA-b and -c transcription. The stepwise transcription of mlonRNAs induces histone acetylation and chromatin remodeling; subsequently, it also facilitates Rst2 binding at UAS2 and massive *fbp1* expression. (C) Schematic representation of the *ade6*-M26 meiotic recombination hotspot. In vegetative growth conditions, *ade6* mRNA is transcribed; in sporulation conditions, a lncRNA (M26 lncRNA) is transcribed from the M26 nonsense mutation point. Histone acetylation and chromatin remodeling are induced around the nonsense mutation and its 3′ region. Double-strand breaks are introduced at multiple sites surrounding this mutation point and meiotic recombination is activated.
tion, cascade transcription of shorter mlonRNAs (mlon-
RNA-b and -c), which is initiated from the region between
UAS1 and UAS2, triggers stepwise chromatin remodeling
in the 5' to 3' direction in the fbp1 promoter; this subse-
sequently facilitates the binding of transcription factor Rst2
at UAS2 and fbp1 gene expression (Fig. 2B) (Hirota et
al., 2008a). In response to glucose starvation, the bind-
ing of transcription factor Atf1 to UAS1 further facilitates
cascade transcription of mlonRNAs and chromatin open-
ing. An experiment involving the insertion of a transcrip-
tional terminator at the fbp1 promoter demonstrated that
RNAPII passage along the fbp1 promoter is required for
chromatin remodeling (Hirota et al., 2008a). In addition,
this mlonRNA transcription-induced chromatin remodel-
ing requires histone acetylation mediated by the histone
acetyltransferase Gcn5 (Takemata et al., 2016) and sub-
sequent chromatin remodeling by the chromatin remod-
elers Snf22 and Hrp3 (Adachi et al., 2018). Although
mlonRNA transcription overlaps with the fbp1 ORF,
mlonRNA-mediated histone modification and chromatin
remodeling are limited to within a region approximately
290 bp from the mlonRNA initiation sites (Senmatsu
et al., 2019). During SER3 repression in S. cerevisiae,
SRG1-mediated chromatin assembly also occurs in a lim-
ited range within approximately 200 bp (Hainer et al.,
2011). Thus, this transcription of IncRNA may act as a
short-range inducer in IncRNA transcription-mediated
chromatin modification. In glucose-rich conditions, an
antisense IncRNA (fbp1-as) is also transcribed from the
3' end of the fbp1 ORF, and the expression pattern of
fbp1-as shows a negative correlation with fbp1 sense
transcripts (mlonRNA-b, mlonRNA-c, and mRNA) (Oda
et al., 2015). A biological understanding of the effect of
fbp1-as expression on fbp1 sense transcription has yet
to be clarified. RNA-seq data analysis indicates that
several transcripts in S. pombe exhibit similar features
to mlonRNAs in fbp1 in their expression pattern: these
IncRNAs are transcribed in a region far upstream of
glucose starvation-activated genes in an Atf1-dependent
manner (Oda et al., 2015). These observations sug-
gest that RNAPII transcription of IncRNAs plays gen-
eral roles in the regulation of genome functions through
modulation of chromatin modification and configuration
in S. pombe. Furthermore, mlonRNA molecules also
affect Atf1 binding at UAS1 upstream of fbp1 in a cis-
acting manner by interacting with the global corepress-
sors Tup11 and Tup12 and thereby counteracting their
repressive functions (Takemata et al., 2016). Tup11/12
are homologs of Drosophila Groucho and S. cerevisiae
Tup1 (Chen and Courey, 2000; Smith and Johnson, 2000),
and they are involved in the repression of fbp1 (Janoo et
al., 2001; Hirota et al., 2003). Moreover, Tup11/12 are
required for the stress-specific response in the chromatin
configuration changes of fbp1 (Hirota et al., 2004). The
repressive function of Tup11/12 is known to be counter-
acted by RNAPII transcription of mlonRNA (Asada et al.,
2015, 2017). Considering these findings, both RNAPII
transcription of mlonRNA and the transcribed molecules
themselves may be involved in fbp1 regulation through
the modulation of chromatin configuration and through
transcription factor binding, respectively (Hirota et al.,
2008a; Takemata et al., 2016).

Similar to the interaction between mlonRNA molecules
and the corepressors Tup11/12, transcription factor YY1
(Yin Yang 1) interacts with proximal nascent IncRNAs in
murine embryonic stem cells (Sigova et al., 2015). Sigova
et al. (2015) suggested that IncRNAs produced in the
vicinity of enhancer and promoter elements capture YY1
transcription factor to regulate gene expression. Thus,
nascent IncRNA transcripts can recruit and trap gene
regulatory factors and act in cis for gene regulation.

The phosphorylation pattern of the RNAPII C-terminal
domain is changed during transcription of mRNA and
IncRNA. This change in phosphorylation pattern is
related to the mRNA processing of RNAPII surveillance
and the recruitment of export factors (Kim et al., 2010;
Tietjen et al., 2010; Milligan et al., 2016). Histone modi-
fication factors, namely histone methyltransferases Set1
(Ng et al., 2003) and Set2 (Li et al., 2002, 2003; Xiao
et al., 2003) or histone acetyltransferases p300 and PCAF
(Cho et al., 1998; Obrdlik et al., 2008), interact with RNA-
PII and their interactions depend on the phosphorylated
form of the RNAPII C-terminal domain. mlonRNA-
transcribing RNAPII exhibits a unique property whereby
the RNAPII induces histone acetylation and chromatin
remodeling (Hirota et al., 2008a). Movement of histone
modification factors through the passage of RNAPII is
one possible mechanism underlying this chromatin regu-
lation by mlonRNA-transcribing RNAPII. Thus, studies
suggest that IncRNA-transcribing RNAPII plays a key
role in chromatin regulation as a "pioneer polymerase"
that initially transcribes across closed chromatin to open
up chromatin configuration.

**IncRNA TRANSCRIPTION-COUPLED
CHROMATIN REMODELING PROMOTES
MEIOTIC RECOMBINATION IN S. POMBE**

Similar to the role of mlonRNA transcription in fbp1
activation, IncRNA transcription also plays a critical role
in the induction of meiotic recombination in ade6-M26,
a well-characterized meiotic recombination hotspot in S.
pombe (Schuchert and Kohli, 1988; Ponticelli et al., 1998;
Hirota et al., 2008b). ade6-M26 is a nonsense mutation
created in the ade6 gene, and this mutation concurrently
creates an Atf1 binding site (Kon et al., 1997). The
5'-truncated transcript (M26 IncRNA) is induced from the
site of the ade6-M26 nonsense mutation during meiosis in
an Atf1-dependent manner; subsequently, histone
acetylation and chromatin remodeling are induced
around the nonsense mutation and the 3’ region (Mizuno et al., 1997; Yamada et al., 2004) (Fig. 2C). Double-strand breaks are induced at multiple sites surrounding the resultant open chromatin region and meiotic recombination is activated (Steiner et al., 2002). Similar to the mlonRNA transcription-mediated chromatin remodeling in fbp1, histone acetyltransferase Gcn5 and chromatin remodeling factors Snf22 and Hrp3 are also involved in this process (Hirota et al., 2008b). Taken together, these findings suggest that lncRNA transcription-mediated chromatin modulation plays various roles in the regulation of wider chromosome functions including transcription and recombination.

**IMPRINTED SILENCING OF Igf2r BY TRANSCRIPTION OF AN ANTISENSE lncRNA (Airn) IN MAMMALIAN CELLS**

Gene regulation mechanisms mediated by lncRNA transcription have also been observed in mammalian cells. A typical example is transcription of the antisense lncRNA Airn (antisense Igf2r RNA noncoding), which overlaps the mammalian imprinted Igf2r gene ORF and promoter and is required for paternal Igf2r silencing (Fig. 3A) (Latos et al., 2012). Igf2r encodes insulin-like growth factor 2 receptor and is silenced in the paternally inherited chromosome due to genome imprinting (Willison, 1991; Stöger et al., 1993). In an experiment in which Airn transcription was terminated by insertion of a polyadenylation (polyA) signal sequence, it was shown that passage of RNAPII through the Igf2r promoter but not Airn lncRNA products is required for the imprinted silencing of Igf2r (Latos et al., 2012). The Igf2r promoter repressed by Airn transcription exhibits some of the features of active chromatin: DNase I hypersensitivity and a high level of H3K4-me3. Hence, Latos et al. (2012) suggested that Airn transcription inhibits functional RNAPII recruitment at the Igf2r promoter possessing an active promoter configuration (Fig. 3A) (Latos et al., 2012). This lncRNA Airn also silences genes in the Igf2r cluster, but how non-overlapping imprinted genes in this cluster are silenced had not been elucidated. Andergassen et al. (2019) recently demonstrated that the Igf2r cluster region has a chromosomal conformation that brings the Airn gene body and the promoters of other genes into close proximity, and expressed Airn lncRNA molecules recruit repressive histone modifiers and target them to the promoters of genes in the Igf2r cluster.

**REGULATION OF Hand2 BY TRANSCRIPTION OF A PROMOTER-ASSOCIATED lncRNA (Uph) IN MAMMALIAN CELLS**

Hand2 encodes a transcription factor required for heart development. Transcription of a promoter-associated lncRNA, referred to as Uph (upperhand), through the region upstream of Hand2 regulates neighboring Hand2 gene expression (Anderson et al., 2016) (Fig. A).

![Fig. 3. Gene regulation by lncRNA transcription in mammalian cells. (A) The lncRNA Airn is transcribed from within the mammalian imprinted Igf2r gene open reading frame and in the opposite direction to Igf2r transcription. The RNAPII (orange) that transcribes Airn moves along the region covering the Igf2r gene promoter and inhibits functional RNAPII (blue) recruitment. (B) The lncRNA Uph is transcribed from a position upstream of the Hand2 gene in the opposite direction to Hand2 transcription. This lncRNA transcription covers the Hand2 promoter and enhancer regions. Uph transcription also maintains active enhancer marks, H3K4-me and H3K27-ac, in the Hand2 promoter and enhancer.](image-url)
Although the Uph sequence is not conserved in mammalian cells, transcription of a Uph-like lncRNA is common in the Hand2 promoter. Transcriptional termination of Uph by the insertion of a polyA sequence causes a defect in Hand2 expression and a phenotype similar to Hand2 knockout; the result is right ventricular hypoplasia and embryonic lethality in mice (Anderson et al., 2016). However, knockdown of Uph mature products has no effect on Hand2 expression. These results indicate that RNAPII transcription of Uph, rather than the Uph transcripts themselves, is important for the expression of Hand2. Premature termination of Uph transcription reduces active enhancer signatures, H3K4-me and H3K27-ac, and binding of GATA4 (a transcription factor required for Hand2 activation in mouse E10.5 embryos) without affecting RNAPII recruitment to the Hand2 initiation site. Therefore, Anderson et al. (2016) suggested that Uph transcription controls Hand2 expression in cis by facilitating the introduction of active enhancer marks following GATA4 binding in the Uph–Hand2 enhancer region.

THE IMPORTANT ROLE OF NASCENT lncRNA TRANSCRIPTION AND SPlicing IN THE REGULATION OF NEIGHBORING GENE EXPRESSION

Engreitz et al. (2016) revealed that nascent lncRNA transcription and subsequent RNA splicing are involved in the regulation of neighboring genes. They analyzed 12 loci in mouse embryonic stem cells and found that five of these loci influenced neighboring gene expression in cis. One such locus was the gene Sfmbt2, at which the lncRNA Blustr is transcribed from the region upstream of Sfmbt2 in the opposite direction to Sfmbt2 transcription. Deletion of Blustr exons or introns diminishes the level of mature Blustr transcript but has no effect on Sfmbt2 activation, indicating that the mature Blustr transcript is not involved in this regulation. In addition, both termination of lncRNA transcription and deletion of the 5′ first splice site of Blustr critically affect Sfmbt2 induction, indicating that nascent Blustr transcription and splicing are involved in Sfmbt2 activation. Because splicing and transcription are closely related (McCracken et al., 1997; Pong and Zhou, 2001; Alexander et al., 2010) and splicing inhibition affects histone modification (H3K36me3) (de Almeida et al., 2011; Kim et al., 2011), splicing machinery coupled with nascent lncRNA may function as a lncRNA transcription-mediated chromatin regulator. Furthermore, RNA–protein interaction analyses have indicated that nascent transcripts interact with some histone modification factors, histone methyltransferases Set1 and Set2 (Battaglia et al., 2017; Sayou et al., 2017), and EZH2 (the catalytic subunit of PRC2) (Kaneko et al., 2013). These observations suggest that nascent lncRNAs act as important scaffolds to recruit regulatory proteins including splicing apparatus and histone modification factors and that they play a pivotal role in the regulation of chromatin geometry in cis (Kaneko et al., 2014; Wei et al., 2016; Skalska et al., 2017).

CONCLUSION

In this review, we summarized the lncRNA transcription-coupled regulation of gene expression that has been identified in budding yeast, fission yeast and mammalian cells. It is possible that numerous human lncRNAs that have been associated with developmental processes (Sigova et al., 2013) and diseases such as cancer (Iyer et al., 2015) are also associated with the regulation of genome function through chromatin modulation. Developing our understanding of the regulation mechanisms underlying such lncRNA transcription-mediated gene control should also increase our understanding of lncRNA-associated biological processes.

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