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Review Article

Long non-coding RNAs: versatile master regulators of gene expression and crucial players in cancer

Lei Nie[1], Hsing-Ju Wu[2], Jung-Mao Hsu[1], Shih-Shin Chang[1], Adam M LaBaff[1], Chia-Wei Li[1], Yan Wang[1], Jennifer L. Hsu[1,2,4], Mien-Chie Hung[1,2,3,4]

[1]Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030; [2]Center for Molecular Medicine, China Medical University Hospital, Taichung, Taiwan; [3]Graduate Institute of Cancer Biology, College of Medicine, China Medical University, Taichung, Taiwan; [4]Asia University, Taichung, Taiwan

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Abstract: With rapid development of sequencing technologies such as deep sequencing and whole genome high-density tiling array, we now know that most of the "junk" genomic sequences are transcribed as non-coding RNAs (ncRNAs). A large number of long ncRNA transcripts (> 200bp) have been identified, and these long ncRNAs (LncRNAs) are found to be crucial regulators for epigenetic modulation, transcription, and translation. In this review, we briefly summarize the regulatory function of LncRNAs with a particular focus on the underlying mechanisms of LncRNAs in oncogenesis, tumor metastasis and suppression.

Keywords: Long non-coding RNA (LncRNA), epigenetic regulation, competitive endogenous RNA (ceRNA), oncogenic lncRNA, pseudogene transcript, natural antisense RNA (NAT)

Introduction

When the whole human genome was determined, it was a surprise that there are only about 20,000-25,000 protein-coding genes, representing less than 2% of whole human genome. It was hard to imagine that most of the genomic sequences are junk DNA since simple organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* have a very close number of protein-coding genes. The limited number of protein-coding genes cannot explain the developmental and physiological complexity of humans. With the rapid development in high-throughput sequencing technologies such as deep sequencing and whole genome high-density tiling array, it is now known that about 98% of the "junk" DNAs are transcribed as non-coding RNAs (ncRNAs) including short and long ncRNAs [1, 2].

A large variety of ncRNAs can be divided into two classes: structural and regulatory ncRNAs (**Table 1**). Structural ncRNAs include tRNA, rRNA, and snoRNA. Based on ncRNA length, regulatory ncRNA can be further divided into at least three groups: (1) Short ncRNA including MicroRNA (miRNA) (22-23 nts) and piwi-interacting RNA (piRNA) (26-31 nts); (2) medium ncRNA (50-200 nts); (3) long ncRNA (>200 nts). This review will focus on medium and long ncRNAs, which are collectively referred to as large or long ncRNAs (LncRNAs). Based on large-scale sequencing and prediction from chromatin-state maps of full length cDNA libraries in FANTOM2 and 3 as well as human transcriptomes, more than 4,600 LncRNAs in mouse and over 3,300 LncRNAs in human have been identified with a total of approximately 23,000 LncRNAs in a mammalian genome [3-7].

Biogenesis of LncRNAs is quite complicated. In general, LncRNA transcription and processing is very similar to protein-coding RNA. Most of LncRNAs are transcribed by RNA polymerase (RNAP) II, but some LncRNAs have been reported to be transcribed by RNAP III, and the majority of LncRNAs are spliced, polyadenylated

Table 1. RNA Classifications

Class	Functions
mRNAs	**Encoding proteins**
ncRNAs	
1. House-keeping or Structural ncRNAs	
tRNA (transfer RNA)	mRNA translation
rRNA (ribosomal RNA)	mRNA translation
snoRNA (small nucleolar RNAs)	rRNA modification
snRNA (small nuclear RNA including spliceosomal RNAs)	RNA splicing, polyadenylation
2. Regulatory ncRNAs	
Short ncRNA	
miRNA (22-23nt)	Degradation of mRNA or repression of translation
piRNA (26-31nt, piwi-interacting RNA)	Silencing of transposons
Medium ncRNA (50-200nt)	
paRNA (promoter-associated ncRNA)	Gene repression *in cis* via interacting with PRC2
Long (large) ncRNA (>200nt)	
Intergenic ncRNA	Epigenetic regulators of transcription *in cis*/*in trans*
Intronic ncRNA	*Ibid*
UTR LncRNA	*Ibid*
Antisense transtcript	mRNA stability of its homologous coding gene
Pseudogene transcript	Generation of NATs or ceRNAs, stabilization of its coding transcript by competitive binding miRNAs
Enhancer-like ncRNA (eRNA)	Activation of promoter activity by unknown mechanism
Mitochondrial ncRNA	Cell cycle regulation and more unknown functions
Repeat-associated ncRNA	Regulation of repeat silencing
Satellite ncRNA	Involvement of formation and function of centromere-associated complexes

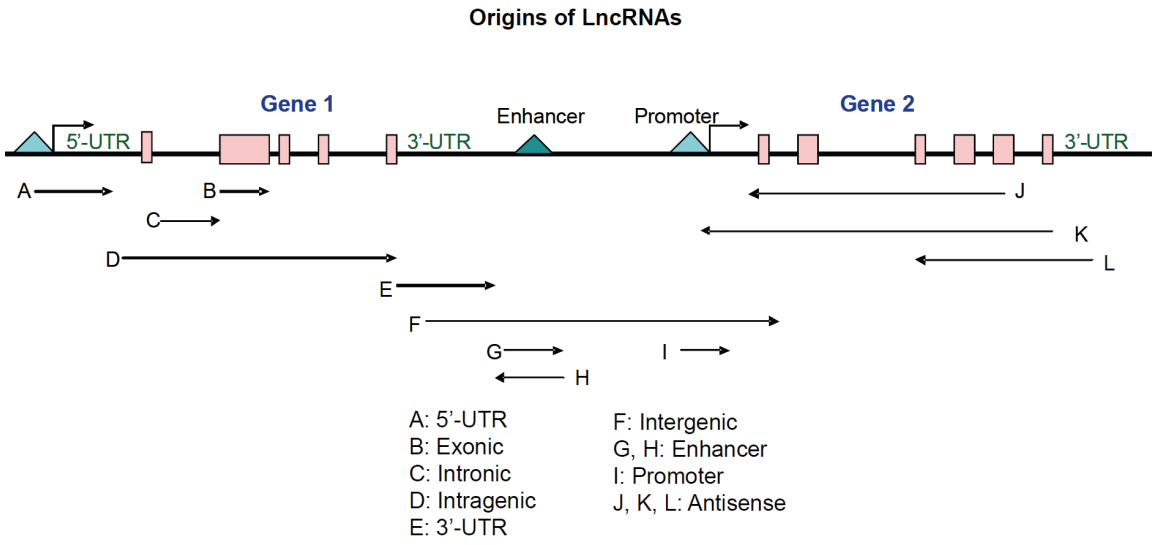

Figure 1. Origins of LncRNA. Arrows represent different types of LncRNA transcripts.

and 5′-capped. Some LncRNAs are evolutionarily conserved and can be expressed at low level. A large proportion of LncRNAs has highly conserved proximal promoter sequence, exonic sequences, intronic sequences or secondary RNA structures. LncRNAs originate from in-

Table 2. Identified tumor and disease-associated LncRNAs

Name	Function/Characterization	References
H19	Imprinted at the Igf2 locus; controls igf2 expression *in cis*, implicated in both tumor suppressors and oncogenes	Maher, ER. et al., 2000 [94]; Gaston, V. et al., 2001 [95]
HOTAIR	Intergenic transcript of HoxC locus, gene silencing in trans through interacting with PCR2 and LSD1 complex, involved in breast cancer metastasis	Tsai, MC. et al., 2010 [15]; Gupta, RA. et al., 2010 [16]; Rinn, JL. et al., 2007 [28]
AIR	Imprinted, monoallelically expressed from the paternal allele, interacts with histone methyltransferase G9a	Wutz, A. et al., 1997 [24]; Nagano, T. et al., 2008 [25]; Sleutels, F et al, 2002 [27]
ANRIL	Antisense transcript of INK4n/ARF/INK4a and p15/CDKN2B, required for the PRC2 recruitment to and silencing $p15^{INK4b}$ tumor suppressor gene	Pasmant, E. et al., 2007 [29]; Yap, KL. et al., 2010 [31]; Kotake, Y. et al., 2011 [32]
HOTAIRM1	Antisense intergenic RNA myeloid 1, transcribed antisense to the HOXA genes, plays a role in the myelopoiesis through modulation of gene expression in the HOXA cluster	Zhang X., et al., 2009 [140]
KCNQ1OT1	Tissue-specific imprinted genes within the Kcnq1 domain, interacting with both PRC2 and G9a leading to gene silencing in a lineage-specific manner	Kanduri, C. et al., 2006 [37]; Pandey, RR. et al., 2008 [33]; Lee, MP. et al., 1999 [97]; Nakano, S. et al., 2006 [98]
Evf-1	Activates transcriptional activity by directly influencing Dlx-2 activity	Kohtz JD & Fishekk G., 2004 [141]
Evf-2	An alternatively spliced form of Evf-1 activates transcriptional activity by directly influencing Dlx-2 activity	Feng, J. et al., 2006 [49]
P15AS	Antisense transcript of p15, highly expressed in leukemia, epigenetically silences the tumor suppressor gene p15	Yu, W. et al., 2008 [107]
Xist	Mosaic expression, spreads on Xi *in cis*, interacts with BRCA1, correlated with breast cancer, cervical, ovarian, and testis tumors	Zhao, J. et al., 2008 [19]; Weakley SM et al. 2011 [80]
Tsix	Antisense transcript to Xist , prevents Xist stabilization and inhibits the interaction between Rep A and PRC2, silencing Xist expression	Zhao,J. et al., 2008 [19]; Navarro, P. et al., 2005 [22]
Zeb2NAT	Antisense to Zeb2, regulates splicing of the IRES-containing intron of Zeb 2, involved in EMT	Beltran, M. et al., 2008 [60]
MALAT-1/ NEAT2	Expressed in many cancers, regulates alternative splicing of pre-mRNA and promotes cell motility through transcriptional and post-transcriptional regulation of motility related gene expression	Tripathi, V., 2010 [66]
MEG3	Imprinted transcripts, highly expressed in human pituitary, stimulates p53-mediated transactivation and suppresses tumor growth in the absence of p53	Gejman, R. et al., 2008 [108]; Benetatos, L. et al., 2011 [110]; Zhou, Y. et al., 2007 [111]
GAS5	Growth arrest-specific transcripts, controls apoptosis and cell cycle, down-regulated in breast cancers	Mourtada-Maarabouni, M. et al., 2008 [114]; Mourtada-Maarabouni, M. et al., 2009 [115]
PCGEM1	Prostate tissue-specific and prostate-associated, overexpressed in prostate cancers, regulates cell proliferation and apoptosis, promotes colony formation	Srikantan, V. et al., 2000 [76]
UCA1	Urothelial carcinoma-associated transcript, upregulated in bladder carcinoma and embryo, influences cell growth and promotes invasion	Wang, F. et al., 2008 [142]; Wang, XS et al., 2006 [143]
SRA-1	Alternative splicing of SRA-1, loss of coding frame, an increased expression is associated with tumor metastasis	Yao, H et al., 2010 [144]
CUDR	Upregulated in drug-resistant human squamous carcinoma, regulates drug sensitivity, cellular transformation and apoptosis	Tsang, WP et al., 2007 [145]
VL30-1	A mouse noncoding retroelement RNA, binds and releases PSF from a proto-oncogene, thus activating Rab23 proto-oncogene transcription	Li, L et al., 2009 [146]; Wang, G. et al., 2009 [147]
Myc	Antisense transcript to myc gene, may be targeting the sense transcripts for immediate degradation	Yu, W. et al., 2008 [107]
p21NAT	Antisense to cdkn1a/p21, requires Ago1 for epigenetic silencing of Cdkn1a/ p21	Yu, W. et al., 2008 [107]
CCND1/ Cyclin D1	Transcribed from 5' end of Cyclin D1 gene, induced by DNA damage and binding to TLS protein, leading to allosteric changes and repression of Cyclin D1 and anti-sense transcripts of tie-1 related to vascular malformation	Wang, X. et al., 2008 [52]
PTENP1	Transcript of PTEN tumor suppressor pseudogene, PTENP1 3'-UTR exerts a tumor suppressive function by acting as a decoy for PTEN-targeting miRNAs	Poliseno, L et al, 2010 [67]; He, L. 2010 [68]
KRAS1P	Transcript of KRAS pseudogene, overexpression of KRAS1P 3'-UTR, increases KRAS mRNA abundance and accelerates cell growth	Poliseno, L et al, 2010 [67]
DHFR	Transcribed from upstream of DHFR gene, regulates DHFR expression by forming triple helix with the promoter and disassociating pre-initiation complex	Martianov, I. et al., 2007 [53]
TERRA	Telomeric UUAGG repeat-containing RNA, inhibits telomerase activity, also regulates Xist and HOTAIR	Azzalin, CM. et al., 2007 [42]; Schoeftner, S. and Blasco, MA., 2008 [44]; Luke, B. and Lingner, J., 2009 [120]
AK023948	Antisense transcribed from the intron of SIR-like adaptor gene (SLA), significantly downregulated in most of papillary thyroid carcinoma	He, H et al., 2009 [148]
TUG1	Ubiquitously expressed in human and mouse cell types and tissues, involves eye development, upregulated by p53, repressed cell proliferation via bound to PRC2	Zhou, Y. et al., 2007 [111]; Huarte, M. et al., 2010 [113]
LincRNA ROR	Expressed in the induced pluripotent stem cells (iPSCs), involved in the conversion of lineage-committed cells by interacting with reprogramming complexes	Loewer, S. et al., 2010 [149]
HAR1	REST target gene, decreased in the neurons of Huntington's disease	Tsai, MC. et al, 2010 [15]
Tie-1AS	Expressed temporally and spatially in vivo with its native gene tie-1, binds tie-1 mRNA, regulating tie-1 transcripts; imbalance of sense	Li, K. et al., 2010 [61]
BACE1AS	Antisense transcript for beta-secretase-1, directly implicated in the increased abundance of Abeta 1-42 in Alzheimer's disease	Faghihi, MA. et al., 2008 [62]; Jiang, Y. et al., 2010 [63]

tronic, exonic, intergenic, intragenic, promoter regions, 3′- and 5′-UTR, and enhancer sequences and are sometimes bidirectional transcripts (**Figure 1**). In particular, a large group of LncRNAs is antisense to known protein-coding transcripts that are also referred to as natural antisense transcripts (NATs) [6, 8, 9]. NATs are divided into two subtypes: cis-NATs, which are transcribed from opposite DNA strands at the same genomic loci; and trans-NATs, which are transcribed from distal loci (**Figure 1**). More recently, emerging experimental evidence revealed that NATs can also be generated from pseudogenes [10, 11]. Notably, many cancer-relevant genes, particularly tumor suppressor genes, produce long antisense ncRNAs (**Table 2**).

Unlike mRNA and structural ncRNAs, most LncRNAs are localized in the nucleus. Some of LncRNAs are found in both cytoplasm and nucleus. However, some LncRNAs are specifically distributed in cytoplasm [12, 13]. Below, we will discuss various LncRNAs with respect to their roles in epigenetic regulation, transcriptional and posttranscriptional regulation as well as tumorigenesis.

Diverse regulatory functions of long non-coding RNAs (LncRNAs)

LncRNAs were initially thought to be spurious transcriptional noise due to low RNA polymerase fidelity. In recent years, it has become evident that LncRNAs are pervasively transcribed throughout eukaryotic genomes. The expression of many LncRNAs occurs in the developmental stage and is cell type-specific. Interestingly, a large number of LncRNAs are specifically expressed during embryonic stem cell differentiation, pathogenesis or tumorigenesis. Up to date, the functions of only a limited number of LncRNAs have been well characterized such as Xist/RepA, KCNQ1OT1, AIR, HOTAIR, Evf -2, H19, MALAT1, and some natural antisense LncRNAs (NATs). However, there is still a large number of LncRNAs for which their biological significance is not yet identified. Transcription of LncRNAs is now known to regulate the expression of protein-coding genes in close genomic proximity (*in cis* or *cis-acting*) and to target distant transcriptional complexes such as activator or repressors (*in trans* or *trans-acting*) via a variety of mechanisms. In this review we will highlight the role of LncRNAs in gene expression regulation at the level of chromatin modification and transcriptional and posttranscriptional modification. Also in this review, we will focus on the association of the identified LncRNAs with diseases, especially cancers.

Epigenetic regulation

It has been demonstrated that LncRNAs can mediate epigenetic modification by recruiting chromatin remodeling complex to specific chromatin loci. A growing body of LncRNAs has been found to associate with chromatin-remodeling complexes and regulate gene expression. Several studies have shown that more than 20% of 3,300 human LncRNAs are bound by Polycomb Repressive Complexes (PRCs) or other types of chromatin remodeling complexes, e.g. SRA by SRC1, ANRIL by PRC1, Xist/RepA by PRC1 and PRC2 complexes, HOTAIR by PRC2 complex and/or LSD1, Air and Kcnq1ot1 by G9a, and CCND1/Cyclin D1 by translocated-in-liposarcoma (TLS)/histone acetyltransferase (HAT) complex (**Figures 2** and **3**) [14-20]. Accumulating data suggest that nuclear LncRNA molecules selectively interact with the components of chromatin-remodeling complexes directly or indirectly such as EZH2, SUZ12, CBX7, CoREST, and JARID1C/SMCX. Here, we will discuss how some of these LncRNAs mediate gene silencing or activation through their interaction with chromatin-remodeling complexes (**Figure 2**).

Xist/RepA

X-inactive specific transcript (Xist) is one of the first identified and best studied LncRNAs. It is a spliced and polyadenylated LncRNA with a size of 17 kb in mouse and 19 kb in human and only expressed in an inactive but not in the active X-chromosome of the female placental mammals [17, 18]. Recent studies found that besides Xist, other LncRNAs including Tsix and Jpx are also involved in the X-chromosome inactivation (XCI) with Xist being the master regulator of the XCI [17-19, 21-23]. In addition, a recent study identified a new 1.6 kb A-rich ncRNA transcript, termed repeat A (RepA), which is transcribed from Xist gene driven by an internal promoter and is comprised of the overlapping sequences of the 5′-end of Xist transcript [19]. RepA initially recruits PRC2 to the X-chromosome through interaction with EZH2 (**Figure 2**). Furthermore, depletion of RepA abolished full-length Xist in-

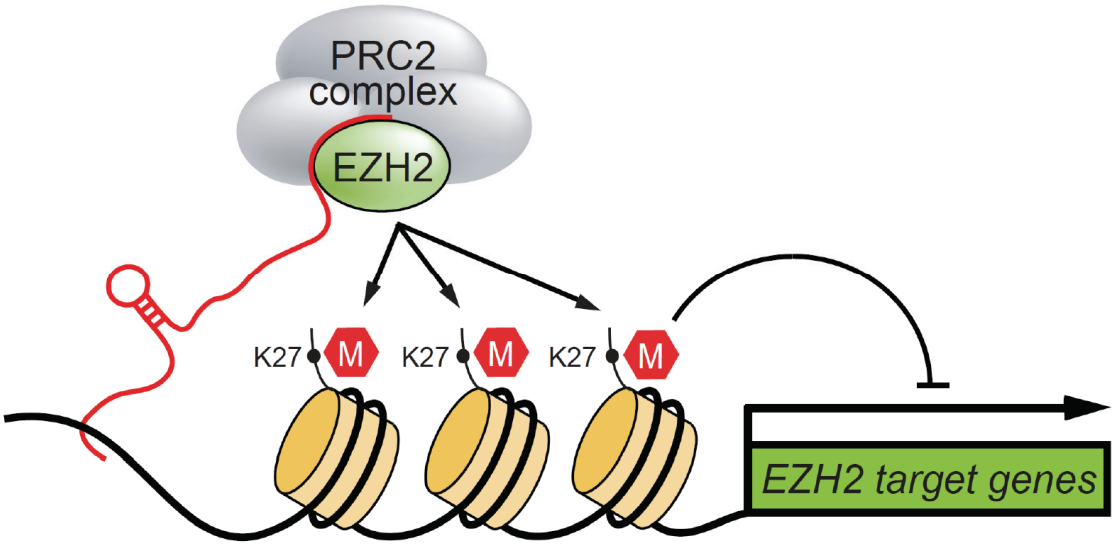

Figure 2. LncRNA in chromatin-remodeling. LcnRNA such as Xist/RepA, Air, Hotair, Tsix, ANRIL and Kcnq1ot1 can recruit Polycomb Repressive Complex (PRC) via direct interaction with EZH2 or other components to the targeted locus where they promote trimethylation of H3K27, leading to silencing of the specific genes.

duction and H3K27 trimethylation of X chromosome, and knock-down of PRC2 subunit Eed and EZH2 compromised Xist upregulation [19]. In addition, Kaneko et al. also showed that like HOTAIR, RepA binds to the N-terminus containing Thr 345 of EZH2 protein, which is defined as the ncRNA binding domain for the 5′-end of RepA/Xist and HOTAIR [21]. The promoter of Xist is repressed by a 40 kb antisense Tsix LncRNA, which also binds to PRC2, and thus inhibits the interaction between RepA and PRC by competing with RepA recruited to pre-XCI [21]. Tsix expression is turned on in both X chromosomes prior to initiation of XCI. At the onset of XCI, Tsix expression turns into monoallelic and is only associated with the future active X-chromosome until Xist expression is off. Tsix does not exist on the inactive X-chromosome once cells undergo the X-inactivation process [19, 21]. Taken together, both RepA and PRC2 are required for the initiation and spread of XCI. The mechanism of how Xist is regulated remains unclear but likely involves negative and positive regulators. For the active X-chromosome, Tsix RNA functions as the established Xist repressor. Whereas for the inactive X-chromosome, another XCI-encoded LncRNA, Jpx, identified by Tian et al., functions as Xist activator [23]. Depletion of Jpx by either knock-out or knockdown approach blocks Xist up-regualtion, suggesting Jpx functions *in trans* and *in cis* [23]. In brief, the expression and function of Xist is controlled by two major ncRNA-based switches: Tsix for active X and Jpx for inactive X.

Air and HOTAIR

Antisense to Igf2r RNA (Air) is a 108 kb, polyadenylated, non-coding RNA that transcribed from an antisense promoter located in intron 2 of the Igf2r (insulin-like growth factor type 2 receptor) in the mouse chromosome 17 [20, 24]. Igf2r gene cluster contains 3 imprinted genes: Igf2r, Slc22a2, and Slc22a3. Unlike Igf2r, Slc22a2, and Slc22a3 maternal transcription, the Air ncRNA is only expressed from the paternal allele [25]. Expression of the Air ncRNA results in a "cloud" nuclear pattern over the imprinted DNA locus during embryonic development in the placenta and the adult heart. The expression of unspliced Air is known to be unstable and exclusively localized to the nucleus while spliced isoforms are relatively stable and found in both nucleus and cytoplasm [25-27]. To date, the most characterized function of Air is regulation of genomic imprinting of the Igf2r gene cluster. This epigenetic process has recently been found to involve histone methylation to achieve monoallelic gene expression without altering the genetic sequence. Using the

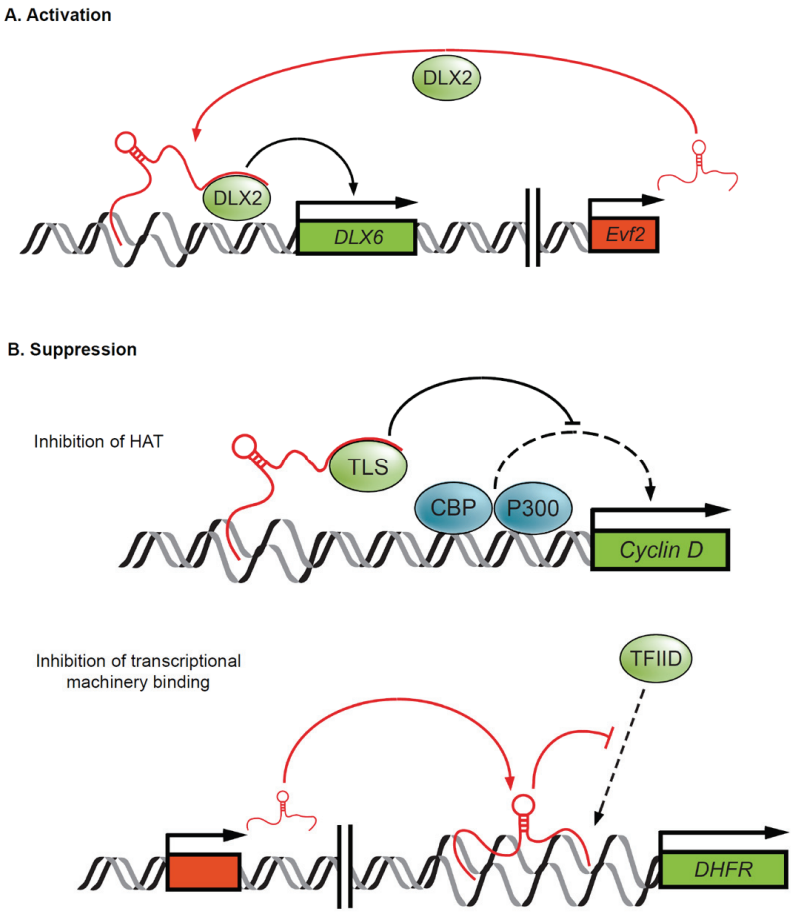

Figure 3. LncRNA in transcriptional regulation. A. Transcriptional activation. LncRNA transcribed from the ultraconserved region of the gene Evf2 functions as coactivator of a homeodomain protein Dlx2, facilitating the Dlx6 gene transcription. B. Transcriptional suppression. Top, LncRNA CCND1 transcribed from the upstream region of the Cyclin D1 gene recruits the RNA-binding protein, termed translocated-in-liposarcoma (TLS), allosterically to modulate HAT activities of CREB-binding protein (CBP) and p300, resulting in inhibition of the gene expression. Bottom, LncRNA transcribed from the dihydrofolate reductase (DHFR) minor promoter can form triplex at its major promoter to prevent the binding of general transcription factors such as TFIID, and subsequently, silence the expression of DHFR.

RNA TRAP (tagging and recovery of associated proteins) technique, Air was found to envelope the Slc22a3 loci in correlation with H3K9 histone methyltransferase (G9a) and transcriptional repression [24]. Genetic ablation of G9a further leads to nonimprinted and biallelic expression of Slc22a3, suggesting that Air recruits G9a to the Slc22a3 promoter, remodels the chromatin structure, and silences monoallelic expression [20, 25, 27]. Although the expression and function of Air LncRNA have been identified in the last decade, the underlying mechanism through which the chromatin remodel complex mediates a direct contact with the Air LncRNA remains unclear.

HOTAIR (HOX Antisense Intergenic RNA) is located at the boundary of two diametrical chromatin domains in the *HOXC* locus. HOTAIR is transcribed antisense to the canonical *HOXC* genes with a size of 2158-nts. This transcript is spliced and polyadenylated. HOTAIR distally regulates the chromosomal domain *in trans* on HOXD locus [28]. Recent study revealed that the 5′ domain of HOTAIR physically interacts with PRC2 methylase and increases its activity, which facilitates histone H3 lysine-27 trimethylation on the HOXD locus and results in silencing of the HOXD gene. RNAi-mediated depletion of HOTAIR dramatically induces the transcriptional activation in HOXD locus with the increased transcripts of HOXD8, HOXD9, HOXD10, and HOXD11 [28]. The 3′ domain of HOTAIR has recently been found to bind with demethylase LSD1 and is required for its occupancy on chromatin and normal function [15]. Therefore, HOTAIR provides a platform for at least two distinct histone modification complexes: a 5′-domain that binds PRC2 and a 3′-domain that interacts with the LSD1/CoREST/REST complex. Tethering two distinct chromatin-remodeling complexes by the HOTAIR scaffold enables RNA-mediated assembly of PRC2 and LSD1, and therefore, coordinates recruitments of both

PRC2 and LSD1 to the chromatin for coupled histone H3K27 methylation and H3K4 demethylation. These results suggest that LncRNAs may function as scaffolds by providing binding surfaces to assemble selected histone modification enzymes, thereby specifying the patterns of histone modifications on target genes (**Figure 2**) [15].

ANRIL

ANRIL is an antisense non-coding RNA transcribed from the INK4 locus. Full- length of ANRIL is polyadenylated and spans 126.3 kb in genome. Within the INK4 locus is a p15/CDKN2B-p16/CDKN2A-p14/ARF gene cluster. The ANRIL gene is deleted along with CDKN2B and CDKN2A in the melanoma-neural system tumor syndrome family [31]. ANRIL is tightly associated with high-risk genetic markers of coronary artery disease (CAD) and is upregulated in prostate cancer [30]. Deletion of the posterior part of ANRIL in the mouse harboring CAD high-risk genetic markers gives rise to substantial suppression of both tumor suppressor genes CDKN2A and CDKN2B [30, 31]. However, the mechanistic insight of the regulation and function of ANRIL is not yet fully understood. Experimental data also revealed that ANRIL is able to bind to chromobox 7 (CBX7) within the PRC1 complexes, leading to repression of the INK4b/ARF/INK4a gene for controlling cell senescence [29]. More recently, Kotake et al. demonstrated that expression of oncogenic Ras, which stimulates the expression of p15INK4B and p16INK4A, inhibits the expression of ANRIL [32]. The authors showed that the p15INK4B locus is bound by SUZ12, a component of PRC2, and is H3K27-trimethylated. Knock-down of ANRIL by shRNA disrupts binding of SUZ12 to the p15INK4B locus and increases the expression of p15INK4B but not p16INK4A or p14ARF, and thus inhibits cellular proliferation. In brief, these results present a working model in which ANRIL binds to and recruits PRC1 or PRC2 to repress the expression of tumor suppressor p15INK4B locus, facilitating oncogenesis.

KCNQ1OT1

Kcnq1ot1 is a 91 kb imprinting LncRNA [33] that is transcribed in the antisense direction to Kcnq1 gene from the paternal mouse chromosome 7 [34, 35] or human chromosome 11. Kcnq1ot1 is an unspliced and polyadenylated transcript and localized exclusively in the nuclear compartment. Several groups [33, 36-39] have demonstrated that Kcnq1ot1 RNA plays important roles in bidirectional silencing in controlling the expression *in cis* of neighboring genes, and such paternally gene repression of the Kcnq1-imprinted domain regulated by Kcnq1to1 is linked to the multilayered silencing pathways. In general, the Kcnq1ot1 RNA harbors a highly conserved domain at its 5'-end, which mediates transcriptional silencing through interaction with G9a/PRC2 complexes in a lineage and stage-specific manner (**Figure 2**). Moreover, studies further showed that EZH2 is required for Kcnq1ot1 association along the length of the Kcnq1 cluster, suggesting that EZH2 may mediate spreading of Kcnq1ot1 and/or play a role in higher-order genomic organization [40, 41].

TERRA

Human telomeric DNA is transcribed into LncRNA, termed TERRA. TERRA is a heterogeneous ncRNA ranging in size from ~100 bases up to at least 9 kb that contains UUAGGG repeats and is complementary to the template sequence of telomerase RNA. Its 5′-end contains a 7-methylguanosine (m^7G) cap structure [42-44]. TERRA is exclusively found in nuclear RNA fractions. TERRA can interact with several telomere-associated proteins such as telomere repeat factors 1 (TRF1) and 2 (TRF2), subunits of the Origin Recognition Complex (ORC), heterochromatin protein 1 (HP1), histone H3 trimethyl K9 (H3 K9me3), and some factors in the DNA damage sensing pathways [43-47]. It was shown recently that TERRA localization may be regulated developmentally, and in turn, might be important for orchestrating some aspects of the complex chromosome transactions that occur during cellular differentiation [44, 45]. Several lines of evidence suggest that TERRA may act as a negative regulator of telomerase and thus establish telomere length homeostasis. Indeed, experimental evidence demonstrated that in addition to exerting an uncompetitive mode of inhibition, TERRA acts as a telomerase ligand and a natural potent competitive inhibitor for human telomerase [46]. TERRA contacts the telomerase reverse transcriptase (TERT) protein subunit independently of human telomerase RNA (hTR) [46]. Lieberman and other groups have shown that TERRA RNA can interact di-

rectly with the TRF2 amino-terminal basic glycine/arginine-rich (GAR) domain and ORC1 to form a stable ternary complex, facilitating TRF2 interaction with ORC, which has been implicated in transcriptional silencing, heterochromatin formation, centrosome function, and sister chromatid cohesion [43, 47]. Taken together, TERRA plays a central role in telomere structural maintenance and heterochromatin formation.

Transcriptional regulation

Some ncRNAs were found to directly regulate transcription of their target genes. With advances in DNA sequencing technology, more and more widespread promoter- and enhancer-associated transcripts have been identified from yeast to human transcriptomes.

Promoter-associated ncRNAs (paRNAs)

paRNAs function mostly through RNAi-mediated pathways of gene repression; however, paRNA with a length of 50-200 nts, has been shown to be involved in epigenetic repression of Polycomb (PcG) target genes via an alternate mechanism. The mechanism of such paRNA gene regulation involves transcription of these RNA transcripts from proximal promoter regions that overlap with the genes targeted for regulation by these uncoding RNAs. Gene silencing occurs due to association of the paRNA with and the recruitment of the PRC2 component, SUZ12, to the target gene promoter regions, enhancing H3K27me3 at the target promoters, and thus repressing gene transcription *in cis* [48]. Another case of paRNA controlling transcriptional regulation involves an intergenic region between members of the Dlx/dll homeodomain-containing protein family, Dlx-5/6 [49]. Feng et al. [49] identifed a 3.8 Kb alternatively spliced form of Evf-1 from an ultra-conserved region in Dlx-5/6 and demonstrated that this paRNA Evf-2 is transcribed under Sonic hedgehog (Shh) treatment. Evf-2 associates with Dlx-2 to increase transcriptional activity of the Dlx-5/6 enhancer in a target- and homeodomain-specific manner *in trans* (**Figure 3A**). In these cases, transcription at the gene promoters, rather than the RNA product of transcription, mediates regulation of the overlapping genes *in cis* or distant enhancer/promoter regions *in trans*. The absence of these paRNAs from PcG target genes that are activated during differentiation in embryonic stem cells (ES) suggests the ncRNAs may maintain cells in an undifferentiated status. This principle may be present in other stem cell-related diseases involving PRC2 [50].

Similarly, paRNA Evf-2 also plays a role in development and is essential for the development of interneurons that produce GABA, the major inhibitory neurotransmitter in the brain. The absence or reduction of GABA has been implicated in neurological disorders such as schizophrenia, Tourette's syndrome, epilepsy, and Rett syndrome, an autism spectrum disorder [49]. Taken together, a new mechanism of transcriptional regulation of gene discovered by both Kanhere et al. [48] and Feng et al. [49] in which the paRNA up-regulates enhancer activity of target genes through recruitment of transcriptional factor DLX-2 to form stable complex at the binding sites. Understanding the potential mechanisms that regulate the transcription and processing of these transcripts may provide therapeutic strategies by targeting these paRNA populations in different respective disease states [51].

In contrast, some paRNAs suppress transcription. For example, Wang et al. [52] demonstrated that DNA damage signals induce transcription of heterogeneous LncRNA CCND1/Cyclin D1 from upstream of the CCND1 promoter, and this low copy of LncRNA transcript is associated with the Cyclin D1 gene promoter and RNA-binding protein termed TLS in which they allosterically modulate the activity of RNA-binding protein, TLS. The modified TLS subsequently inhibits the HAT activities of CREB-binding protein (CBP) and p300, leading to silencing of Cyclin D1 expression (**Figure 3B**, top panel). In addition, some paRNAs can regulate RNAP II activity by competing with transcription factors. For example, in humans, the dihydrofolate reductase (DHFR) gene contains a minor and major promoter. The major promoter activity is suppressed in quiescent cells. A LncRNA DHFR transcribed from upstream of the minor promoter binds to both the major promoter of DHFR and the general transcription factor TFIIB, preventing the formation of pre-initiation complex at the major promoter site (**Figure 3B**, bottom panel). DHFR transcript and double-stranded DHFR promoter form a stable purine-purine-pyrimidine triple structure [53]. Triplex structure may be a common LncRNA mechanism for inhibition of targeted promoter activity.

Enhancer-like LncRNAs (eRNAs)

More recently, Ørom et al. [54, 55] used a GENCODE annotation of the human genome to characterize over a thousand LncRNAs with sizes that ranged from 100 to 9100 nts that are expressed in multiple cell lines. Unexpectedly, in contrast to the well-characterized LncRNA such as XIST, they found that some LncRNAs have functional properties of enhancers in human cell lines. Depletion of this type of LncRNAs (eRNAs) led to potent decreased expression of their neighboring protein-coding genes, including the master transcription factors TAL1/SCL, Snail (Snai1) and Slug (Snai2). For example, depletion of ncRNA-a3 significantly decreased the expression of its flanking gene Tal1/SCL, which is a key regulator in hematopoiesis. In addition, knock-down of ncRNA-a7 reduced the expression of its neighboring gene Snai1, which plays a crucial role in cell adhesion, migration, and epithelial-messenchymal transition [54]. These findings demonstrate that such enhancer -like eRNAs display a transcriptional activator function for their neighboring genes, suggesting that an unanticipated role for this class of LncRNAs in activation of critical regulators involved in development and differentiation [54, 55]. Similarly, using the active chromatin marker H3K4 monomethylation, Kim et al. [56] found that enhancer domains can be transcribed by RNAPII bi-directionally to a novel class of eRNAs. Moreover, the eRNA expression level at neuronal enhancers positively correlates with the level of mRNA synthesis at nearby genes. Their solid data demonstrate that eRNA synthesis occurs specifically at the enhancers that are actively involved in promoting mRNA synthesis [56]. The reported evidence indicates that LncRNAs that associates with enhancer regions are important regulators for modulating their neighboring gene expression [54-56].

Post-transcriptional regulation

In addition to the above mechanisms, LncRNAs are also involved in posttranslational processing of mRNAs, including splicing, editing, trafficking, translation, and degradation. They are summarized below:

Naturally occurring antisense transcripts (NATs)

Of the most particular types of LncRNAs, NATs play a very crucial role in regulating mRNA dynamics. It is estimated in human and mouse that 61-72% of all transcribed regions posses LncRNAs in antisense orientation (NATs) [8, 57-59]. Unlike classical NAT LncRNAs to the imprinting genes such as Tsix, Air, HOTAIR, and Evf -2, which guide the epigenetic silencing complexes to the targeted loci, some NATs can form RNA duplexes to mask key cis-regulatory elements in the mRNA of overlapping gene, leading to alternative splicing pattern of the paired gene. For example, the Zeb2/Sip1 NAT is complementary to the 5′ splice site of an intron in the 5′-UTR of the zinc finger Hox mRNA Zeb2, which is involved in epithelial-mesenchymal transition (EMT). Expression of the Zeb2 NAT upon EMT can mask the splice site, preventing splicesome from functioning. As a result, the translation machinery can then recognize and bind to an internal ribosome entry site (IRES) in the retained intron, resulting in more efficient Zeb2 translation (**Figure 4A**) [60]. More recently, Li et al. [61] identified a NAT for tyrosine kinase containing immunoglobin and epidermal growth factor homology domain-1 (Tie-1AS) in zebrafish, mouse, and human. The tie-1 NAT selectively binds tie-1 mRNA *in vivo* to form RNA-RNA duplex, resulting in downregulation of the Tie-1 protein and thus specific defects in endothelial cell contact junctions *in vivo* and *in vitro*. In contrast, the expression of antisense transcript (BACE-1AS) of a crucial enzyme in Alzeimers's disease pathophysiology, Beta-secretase-1 (BACE-1), increases BACE-1 mRNA stability and generates more Abeta (amyloid-beta) 1-42 through a post-transcriptional feed-forward mechanism [62, 63].

LncRNAs affecting pre-mRNA splicing

Higher eukaryotes are frequently observed to use a well-known mechanism termed alternative splicing of pre-mRNA to diversify their transcriptomes and to increase their proteomic complexities. The serine/arginine (SR) splicing factors have been demonstrated to be engaged in regulating tissue- or cell-type-specific alternative splicing in a concentration- and phosphorylation-dependent manner [64-66]. However, the underlying mechanisms that modulate the cellular levels of active SR proteins remain unclear. Recent studies present evidence showing that long nuclear-retained regulatory RNA (nrRNA), referred to as metastasis-associated lung carcinoma transcript 1 (MALAT1), plays a crucial role in alternative splicing regulation [64, 66]. MALAT1, also known as NEAT2 (noncoding nu-

clear-enriched abundant transcript 2), is about 7 kb long and localized in nuclear speckles and interacts with SR splicing factor, SRSF1, which affects the distribution of these and other splicing factors in nuclear speckle domains. Depletion of MALAT1 with antisense oligonucleotides or transient overexpression of SRSF1 changes the alternative splicing of the endogenous pre-mRNAs. Importantly, MALAT1 controls cellular phosphorylation status of SR proteins, thereby regulating cellular ratio of phosphorylated vs. dephosphorylated form of SR proteins [66], suggesting that MALAT1 regulates pre-mRNA processing via modulating the levels of active SR proteins.

Pseudogene transcripts

Recently, over 10,000 pseudogene mRNAs have been identified, accounting for 10% of the FANTOM3 transcriptome [10]. The biological importance of pseudogene mRNAs, however, is less understood. There is a growing body of evidence to demonstrate that pseudogene transcripts play an important role in regulating mRNA stability of its coding paralogue. Interestingly, recent studies revealed that pseudogene transcripts of tumor suppressor gene PTEN (PTENP1) and oncogenic KRAS (KRASP) are biologically active as they can specifically regulate cellular levels of their counterpart genes, PTEN and KRAS [67, 68]. Emerging evidence demonstrated the functional importance of PTEN dosage during tumor development. Among the key regulators of PTEN dosage are a number of noncoding RNAs, including miRNAs and pseudogene transcripts, which regulate PTEN abundance at the posttranscriptional level. Recent investigations unveiled that PTEN and PTENP1 3′-UTR are highly conserved, and PTEN mRNA is protected from common miRNA binding at the microRNA response elements in 3'-UTR region by PTENP1 RNA, which is also called competing endogenous RNA (ceRNA). The competitive binding of ceRNAs to the common miRNAs results in the increase of PTEN mRNA abundance and protein level (**Figure 4B**) [67-72]. Similarly, KRAS mRNA level is also increased by expression of ceRNAs of KRASP [67-69]. Besides pseudogene transcripts, it should be noted that emerging evidence supports the notion that some protein-coding mRNAs also function as ceRNAs. By a combined computational and experimental approach researchers have identified and validated more endogenous ceRNAs [69-72]. Interestingly, Pandolfi's group expanded ceRNAs to include some protein-coding mRNA transcripts such as ZEB2, VAPA, CNOT6L. Like pseudogene transcripts, these types of endogenouse ceRNAs also modulate PTEN protein levels in a microRNA-dependent, protein coding-independent manner [69-70, 72].

Trafficking regulator

LncRNAs have been shown to modulate the activity of proteins by regulating their subcellular localization exemplified by the transcription factor NFAT (nuclear factor of activated T cells). NFAT transcript is localized in the cytoplasm until calcium-dependent signals cause it to be imported into the nucleus, where it activates transcription of target genes. One of the key regulators of NFAT trafficking is NRON (noncoding repressor of NFAT). The NRON gene contains three exons, and it can be alternatively spliced to produce variant transcripts ranging in size from 0.8 to 3.7 kb. By directly binding to importin-beta 1, one of the components of the nucleocytoplasmic trafficking machinery, NRON specifically inhibits the nuclear accumulation of NFAT but not that of other transcription factors such as p53, AP1, the fork-head FOXO1 and NFκB that also translocate from the cytoplasm to nucleus [73].

LncRNAs and cancer

The relevance of LncRNAs in normal physiological processes and pathogenesis, especially in cancer is increasingly recognized. In recent years, different new approaches such as genome-wide gene expression screen, genome-wide association studies, region-targeted association assay and conventional linkage screen, designed LncRNA array, RIP-RNA sequencing as well as transgenic expression and gene knockdown/knockout have all been successfully used to identify the functions of LncRNAs in cancer. Accumulating data shows that many identified LncRNAs are crucial players in a variety of tissue carcinogenesis, invasion, and metastasis [74, 75]. Based on their functions, LncRNAs can be roughly classified into oncogenic and tumor-suppressor groups (**Table 2**).

Oncogenic LncRNAs

It is now known that similar to protein-coding

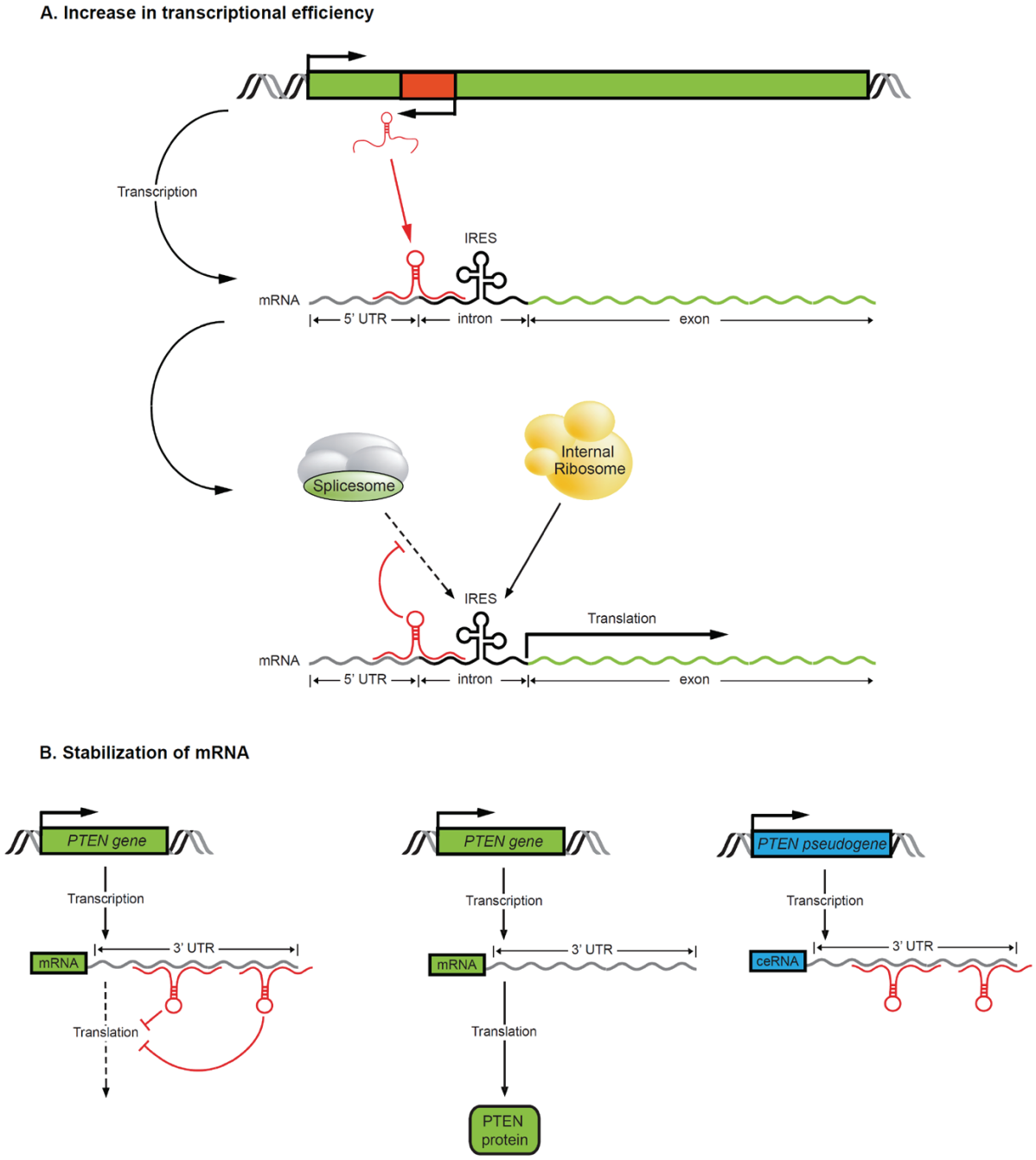

Figure 4. LncRNA in posttranscriptional regulation. A. Increase in translational efficiency. Transcripts of the antisense LncRNA, in this case, NAT Zeb2 can mask the 5′ splice site of an intron in the 5′-UTR of the zinc finger Hox mRNA Zeb2 by complementary interaction, preventing spliceosome binding to generate an inhibitory sequences for ribosome scanning. This results in binding of the translational machinery to an internal ribosome entry site (IRES), leading to more efficient translation of Zeb2. B. Stabilization of mRNA. Transcripts of pseudogenes and its counterpart gene (in this example, PTENP1 and PTEN) are highly conserved at 3'-UTR, such transcripts act as competing endogenous RNA (ceRNA) and thereby the PTEN mRNA is protected from common miRNA (A, B) targeting for degradation by binding with PTENP1 transcripts competitively, as a result, increasing the PTEN mRNA abundance and protein level.

oncogenes, some LncRNAs can also regulate cellular pathways that lead to oncogenesis or metastasis. These types of LncRNAs are referred to as oncogenic transcripts. The first

identified oncogenic or pro-oncogenic LncRNAs are PCGEM1 and PINC, which are highly expressed in prostate and breast cancer [76-79]. Recently, more and more putative onco-LncRNAs have been discovered such as KRASP, HULC, HOTAIR, MALAT1/NEAT1, p15AS, ANRIL, H19, SRA1, p21NAT, and RICTOR (**Table 2**). It is worth noting that some LncRNAs may have oncogenic and/or tumor suppressive effect, depending on the cellular context. For example, XIST transcript is upregulated in some male cancers but downregulated in female cancers, respectively [80]. Here, we will discuss some examples of such LncRNAs.

MALAT-1 LncRNA

Using subtractive hybridization approach, one LncRNA, referred to as cancer metastasis-associated lung adenocarcinoma transcript, MALAT-1, was originally identified in non-small-cell lung cancer [81]. MALAT-1 is an abundant LncRNA of ~8.7 kb and plays a pivotal role in cell proliferation, migration, and invasion. As mentioned above in this review, MALAT-1 predominantly localizes to nucleus, especially in nuclear speckles in a transcription dependent manner to regulate post-transcriptional processing events such as alternative splicing of mRNAs [64-65]. This LncRNA was significantly upregulated in many cancers such as in the lung, breast, prostate, liver, and colon [81-86]. Investigations have provided evidence that much higher expression of MALAT-1 was found in the metastatic tumors than in non-metastatic tumors, and its expression level in early stage of non-small cell lung cancer is closely correlated with poor prognosis [81]. Lin et al. showed that in all of nodules of procarcinogen-induced murine hepatocellular carcinomas (HCCs) and human HCCs, MALAT-1 expression was markedly elevated compared with the normal liver tissues [83]. In addition, the study also found a significant increase in MALAT1 expression in breast, pancreatic, colon and lung cancers compared with the surrounding normal tissues [83]. Therefore, MALAT1 could be a prognostic marker for metastasis and survival of human cancers [81, 83, 85]. Recent study also demonstrated that MALAT-1 is involved in regulating cell mobility as knockdown of MALAT-1 impaired the *in vitro* migration of the lung cancer cells [86]. Furthermore, the researchers also measured the pre-mRNA and mature mRNA levels of some MALAT-1 target genes involved in migration. Their results suggested that MALAT1 regulates its target gene expression at both transcriptional and post-transcriptional level [86]. However, the underlying mechanism of MALAT-1 contributing to tumor metastatic process remains unclear.

Tumor marker LncRNA

Recent genome-wide association study (GWAS) unveiled strong and reproducible associations of multiple genetic variants in a large "gene-desert" region of chromosome 8q24 with susceptibility to prostate cancer (PC). Using fine mapping and re-sequencing approaches combined with single-nucleotide polymorphism (SNP) analysis, a 13 kb intron-less LncRNA, termed PRNCR1 (prostate cancer non-coding RNA1) was identified [87]. PRNCR1 expression was upregulated in some of the PC cells as well as precursor lesion prostatic intraepithelial neoplasia and considered as one of tumor markers. Knock-down of PRNCR1 by siRNA attenuated the viability of PC cells and the transactivation activity of androgen receptor, which indicates that PRNCR1 could be involved in prostate carcinogenesis possibly through androgen receptor activity. These findings could provide a new insight in understanding the pathogenesis of genetic factors for PC susceptibility and prostate carcinogenesis [87].

The experiments performed by Silva JM et al. [88] revealed that many of the Long Stress-Induced Non-coding Transcripts (LSINCTs) were overexpressed in a number of lung and some breast cancer cell lines compared with normal human bronchial epithelial cells or normal breast epithelial cell line and immortalized MCF10 cells. LSINCT transcripts are intergenic and intragenic LncRNAs, ranging from 2 kb to 4 kb in length. Interestingly, the study indicates that some LSINCTs are overexpressed in the $HER2^-$ and $TP53^+$ breast cell lines [88]. By RNA Amplification of cDNA Ends (RACE) and Northern blots, the authors validated these LSINCTs overexpressed in a panel of breast cancer cells. The larger transcript of LSINCT5 is about 2.5 kb in length. LSINCT5 is polyadenylated and transcribed *in trans*. Increased expression of LSINCT5 has been observed in a panel of breast cancer cells. Moreover, several other cancers including cervix and ovary cancers also have increased LSINCT5 expression [88].

Intergenic LncRNA (LincRNA)

LincRNA HOTAIR is expressed mainly in poste-

rior and distal sites and highly conserved during vertebrate evolution [28], and the latest study showed that HOTAIR is also involved in the metastasis of breast cancer [16]. HOTAIR is overexpressed in metastatic breast cancers and predicts poor prognosis. Dysregulation of HOTAIR represses the expression of a set of cell-cell interaction promoting genes including *JAM2*, *PCDH10*, *PCDHB5*, and *EPHA1* and conversely enhances the expression of many metastasis-facilitating genes, including *ABL2*, *SNAIL*, *LAMB3*, and *LAMC2*. Moreover, the function of HOTAIR is counteracted by knock-down of PRC2 [16]. Therefore, the interaction of HOTAIR and PRC2, leading to increased H3K27 trimethylation and silencing of metastasis suppressor genes, is responsible for the HOTAIR-mediated tumor cell invasion and subsequent metastasis. In addition, recent study showed that an increased expression level of HOTAIR transcript in hepatocellular carcinoma (HCC) patients correlates with a significantly shorter recurrence-free survival, suggesting that HOTAIR transcripts in HCC could be a candidate biomarker for predicting tumor recurrence in HCC patients who underwent liver transplant therapy [89]. Moreover, *in vitro* assays demonstrated that knock-down of HOTAIR in liver cancer cell line reduced cell viability and invasion, and sensitized TNF-α-induced apoptosis, resulting in increase of chemotherapeutic sensitivities of the cancer cells to cisplatin and doxorubicin [89]. These findings indicate that lincRNAs have active roles in modulating the cancer epigenome and may be an important predictor for cancer outcome and novel targets for caner therapy.

Imprinted LncRNAs

Abnormal genomic imprinting, in particular, loss of imprinting (LOI) is involved in a number of human hereditary diseases and cancers [90]. Studies have demonstrated that a disruption in the expression of imprinted genes such as H19, $p57^{kip2}$, IGF2, and KvLQT1 in chromosome band 11p15.5 results in approximately 80% of Beckwith-Wiedemann syndrome (BWS). About 5 -10% of BWS patients are predisposed to a variety of childhood tumors, including hepatoblastoma, rhabdomyosarcoma, neuroblastoma, adrenal carcinoma, and Wilms tumor [91-95]. Kcnq1ot1 (also called LIT1) is an imprinted antisense LncRNA within the human KvLQT1 locus, which is about 60 kb long and has a silencing domain at its 5′-end [40]. Kcnq1ot1 transcript is associated with multiple balanced chromosomal rearrangements in BWS and additional breakpoint in embryonal rhabdoid tumors [96]. Kcnq1ot1 is expressed normally from the paternal allele, from which the KvLQT1 transcription is silent. Abnormal expression of Kcnq1ot1 in both the paternal and maternal allele, e.g. LOI, was found in 50% BWS patients and 53% colorectal cancer [96-98]. LOI of Kcnq1ot1 transcript is closely accompanied by loss of methylation (LOM) of maternal allele of the differentially methylated regions (DMR) located in intron 10 of KvLQT1 gene (KvDMR1). Moreover, LOM of the control element CpG island, namely KvDMR1, strongly correlates with loss of H3K9 dimethylation in both of BWS and cancer [96-100]. KvDMR contains the promoter for the paternally expressed Kcnq1ot1. Disruption of the promoter abolishes Kcnq1ot1 transcripts, leading to activation of neighbor silencing imprinted genes, such as CDKN1C, a tumor repressor [36, 37, 99]. It has been found that silencing of the imprinted CDKN1C gene expression is associated with loss of CpG and histone H3 lysine 9 methylation at KvDMR in esophageal cancers [100]. Taken together, these data suggest that abnormal expression of Kcnq1ot1 contributes to carcinogenesis.

Antisense LncRNA

Recently, many studies described consistent and significant differences in the distribution of sense and antisense transcripts between normal and neoplastic tissues. Many of the differentially expressed antisense transcripts likely represent LncRNAs [57-60]. A subset of genes that mainly generate NATs in normal but not cancer cells is involved in essential metabolic processes, and a large body of evidence shows that altered ratio of sense and antisense transcripts contributes to tumorigenesis and cancer progression [101-106]. For instance, Yu et al. found that leukemia cells had larger amounts of p15 NAT (p15AS) and smaller amounts of its partner p15 mRNA than normal lymphocytes [107]. The length of the p15AS transcript is around 3.5 kb. Furthermore, they demonstrated that ectopic expression of p15AS induces p15 silencing *in cis* and *in trans* through heterochromatin formation but not DNA methylation [107]. It is noteworthy to mention that this group also found that many NAT LncRNAs to the cancer-relevant genes exist such as those encoding p21, p53, E-cadherin, myc, Tie-1, p27KIP1, APC,

RB1, NF1, PTEN, CDKN1A, CDKN2A, CDKN2B, BRCA1, BRCA2, VHL, TP63, TP73, ARF, WT1, and MYC [107]. Thus, tumorigenic NATs may be a trigger for heterochromatin formation and DNA methylation in tumor suppressor silencing in tumorigenesis. Interestingly, experimental evidence also demonstrated that NATs are involved in epithelial-mesenchymal transition (EMT) process exemplified by Zeb2 NAT [60]. Beltran et al. showed that the expression of a transcriptional repressor of E-cadherin Zeb2 (also called Sip1, Smad-interacting protein 1) is upregulated after Snail1-induced EMT [60]. Snail1 does not change Zeb2 mRNA level, but alters the processing of a large intron located within its 5′-UTR (**Figure 4A**). Ectopic expression of Zeb2 NAT in epithelial cells prevents splicing of the Zeb2 5′-UTR and increases translation efficiency of Zeb2, resulting in downregulation of E-cadherin. Therefore, Zeb2 NAT plays a crucial role in regulating E-cadherin expression during EMT [60].

Tumor suppressor LncRNAs

Like protein-coding tumor suppressors, some LncRNAs are found to function as tumor suppressors, including MEG3, GAS5, LincRNA-p21, PTENP1, TERRA, CCND1/Cyclin D1, and TUG1.

p53-related LncRNAs

The LncRNA MEG3 is a transcript of a maternally imprinted RNA gene. All normal human pituitary cell types express MEG3, but the loss of MEG3 expression occurs in pituitary adenomas of a gonadotroph origin [108]. In addition, loss of MEG3 expression was also found in the majority of human meningiomas or the human meningioma cell lines [109]. MEG3 was found to be a positive regulator of p53, a tumor suppressor protein. Ectopic expression of MEG3 significantly increases p53 protein level and dramatically stimulates p53-dependent transcription from p53-responsive promoter [110, 111]. Coactivation of p53 is MEG3-transcription -dependent and requires intact secondary structure of the LncRNA. Furthermore, MEG3 selectively enhances p53 binding to its target promoter such as GDF15 but not p21. MEG3 is also able to inhibit cell proliferation in the absence of p53 [108-112]. These data suggest that MEG3 function as a tumor suppressor in both p53-dependent and p53-independent manner.

Another example of tumor suppressor LncRNA is LincRNA-p21, whose expression is directly induced by the p53 signaling pathway. LincRNA-p21 is required for global repression of genes that interfere with p53 function to regulate cellular apoptosis. LincRNA-p21-mediated gene repression occurs through physical interaction with RNA-binding protein hnRNP-K, leading to its localization to the promoters of genes to be repressed in a p53-dependent manner [113].

Receptor-binding LncRNA

Growth arrest-specific 5 or GAS5 is another example of tumor-suppressor LncRNA which plays an essential role in normal growth arrest in lymphocytes. Overexpression of GAS5 causes both an increase in apoptosis and a reduction in the rate of progression through the cell cycle while downregulation of endogenous GAS5 inhibits apoptosis and maintains a more rapid cell cycle, indicating that GAS5 expression is both necessary and sufficient for normal growth arrest in lymphocytes [114]. GAS5 LncRNA regulates the expression of a critical group of genes with tumor suppressive consequences [115]. GAS5 encodes two canonical splice variants. In addition, multiple snoRNAs are solely transcribed from the introns of GAS5. Under starvation conditions, GAS5 is induced and directly interacts with the DNA binding domain of glucocorticoid receptor (GR) through an RNA-sequence mimic of the GR response element (GRE) DNA, leading to inhibition of GR binding at its target gene promoters to induce their transcription. Repression of the transcriptional activity by GRE-mimic binding of GAS5 is not unique to GR, and other members of nuclear receptors are also affected [116]. In breast cancer, GAS5 was found to be significantly downregulated [115].

Promoter-associated LncRNA

In human cell lines, CCND/Cyclin D1, a *cis*-acting heterogeneous LncRNA that is about 200 bp and >330 bp transcripts. These LncRNAs are originated from the promoter region of the CCND1/Cyclin D1 gene. CCND1/Cyclin D1 transcripts have been shown to bind TLS (also termed FU, Fused in Ewing's Sarcoma). The binding of ncRNA to TLS allosterically activates the repressor and tethers the LncRNA to the CCND1 promoter to inhibit CCND1 expression (**Figure 3B**) [52]. Cyclin D1 encoded by CCND1 gene is frequently overexpressed in human tu-

mors, and nuclear Cyclin D1 is oncogenic driver in human cancers [117]. Therefore, CCND1/Cyclin D1 transcript functions as tumor suppressor to repress tumorigenesis.

Pseudogene LncRNA

Some pseudogene transcripts of tumor suppressors possess tumor suppressive effect [67-68]. An exceptional example is PTENP1, a coding-independent pseudogene of the PTEN mRNA. PTENP1 is biologically active and functions as ceRNA, which can positively regulate PTEN protein level, exerting a growth-suppressive role via competitively binding of interfering miRNAs to the 3′-UTR of PTEN for degradation (**Figure 4B**) [67, 69-72]. These findings may lead to development of a novel approach for cancer therapy.

Telomere-related LncRNAs

TERRA expression is highly dependent on developmental status, including nuclear reprogramming, telomere length, cellular stresses, tumor stage, and chromatin structure [43-47]. Misregulated TERRA is responsible for many of the abnormal telomere phenotypes seen in aging and cancer cells [118-120]. Interestingly, TERRAs are significantly downregulated in advanced stages of different types of human cancers compared with normal tissues [44]. Consistently, low levels of TERRA have been observed in the tumor-derived and *in vitro*-immortalized cell lines [121]. These findings suggest that regulation of TERRA level and TERRA-regulated telomere length play important roles in tumor development that involve telomerase misregulation.

Opportunities and challenges

The rapid advances in biotechnology combined with bioinformatics accelerate the progression of genome-wide studies of LncRNA. It has been known to date that a high percentage of the genomic DNA of mammalian cells is transcribed into RNAs, of which most are non-protein coding transcripts. In particular, around 50% of protein-coding RNAs have their paired long antisense ncRNAs [57-59]. These LncRNAs function as key regulators implicated in numerous functions in regulating gene expression pattern, modulating protein activity, altering the RNA processing, serving as structural components and precursors for small RNAs, and influencing mRNA stability. Up to date, only a small portion of LncRNAs has been identified. Like miRNAs and pRNAs, LncRNAs have emerged as important regulatory molecules in developmental, tumor-suppressor and oncogenic pathways and other diseases [69-71, 122-128]. Some of the LncRNAs have become diagnostic markers and potential therapeutic targets [123-125]. Currently, the study of LncRNAs is becoming one of the most popular fields in the biological and medical sciences.

However, an existing challenge is to understand how the molecular functions of LncRNAs affect the phenotypes of the organisms. Limited by technological advances in the identification of the biological function of LncRNAs, siRNA knock-down is currently the only effective approach, and ectopic expression of LncRNA *in trans* does not always recapitulate the function *in cis*. Moreover, some of the LncRNAs are very large in size and can sometimes exceed 8 kb in length with many secondary structures, which present challenges for cloning and ectopic expression. Future work will focus on whether expression of LncRNA itself is enough to produce observable functional effects.

Characterization of interacting domains such as the stem-loop structure of LncRNAs is also imperative for deciphering their biological functions that will provide effective basis for further validation by genetic approaches and for novel drug development. Generation of knockout and/or transgenic mouse model can determine those LncRNAs, which are not transcriptional noise or junk but are required for normal development and pathogenesis. Recently, two LncRNA mouse models have been reported [126-132]. When maintained in a semi-natural environment, knockout mice of BC1, a rodent-specific LncRNA, display global phenotypes such as reduced exploration, increased anxiety, and mortality compared with wild-type [126-128]. The neural phenotype is consistent with the functional absence of a BCL-interacting protein, FMRP, which is the product of the fragile X mental retardation gene. Another special transgenic mouse model of human spinocerebellar ataxia type 8 (SCA8) demonstrated that antisense RNA of the Kelch-like 1 gene (KLHL1) is associated with a dominantly inherited, slowly progressive neurodegeneration disorder SCA8 [129]. Patients with SCA8 show a trinucleotide (CUG) expansion in a noncoding RNA termed

ataxin 8 opposite strand (ATXN8OS), an antisense transcript to the KLHL1 gene [130]. Transgenic mice containing this pathogenic CTG expansion in their DNA, like human patient, exhibit a progressive neurological phenotype in a dose-dependent manner [129]. Tri- or tetranucleotide repeat expansions within ATXN8OS are proposed to form hairpin structures that drive splicing regulators away from their normal pre-mRNA targets [129, 131, 132].

From these studies, it is now clear that LncRNAs are linked to many diseases such as cancer and neuropathy [74, 75, 127, 128]. The implication of LncRNAs in tumorigenesis, metastasis, and progression remain to be further investigated. Unlike protein-coding RNAs, mutations and polymorphisms in LncRNAs are not frequently reported and genome-wide mutagenesis and association studies have yet completed to define the causative changes in LncRNA sequences. So far, only a few studies pinpoint to high-risk alleles within LncRNA genes such as prostate cancers [123]. Expression profiling of LncRNAs during brain development, iPS cell generation, ES cells and lymphocyte differentiation as well as breast cancers have only been recently documented [12-14, 113, 132-139]. Therefore, characterization of oncogenic and tumor suppressor LncRNAs is an attractive field, which will lead to new markers of cancer diagnosis and identification of novel therapeutic targets.

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Address correspondence to: Dr. Mien-Chie Hung, Department of Molecular and Cellular Oncology, Unit 108, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA Tel: 713-792-3668; Fax: 713-794-3270; E-mail: mhung@mdanderson.org

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