Uncovering the role of genomic "Dark Matter" in human disease

Lance Martin[1,2] and Howard Y. Chang[1]

[1]Howard Hughes Medical Institute and Program in Epithelial Biology, Stanford University School of Medicine, Stanford CA 94305.
[2]Dept. of Bioengineering, Stanford University, Stanford, CA 94305.

Correspondence to: H.Y.C. at howchang@stanford.edu

Abstract

The human genome encodes thousands of long noncoding RNAs (lncRNAs). Though many remain functionally un-characterized biological "Dark Matter," a sub-set of well-studied lncRNAs have garnered considerable attention for their diverse roles in regulation of important loci, including developmental and tumor suppressor gene clusters. Because a growing number of lncRNAs are associated with human disease, ongoing research efforts are focused on understanding their regulatory mechanisms. New technologies that enable rapid enumeration of lncRNA protein partners, secondary structures, and genomic binding sites are well positioned to drive deeper understanding of lncRNA regulation and involvement in pathogenesis.

Introduction

RNA is now recognized as a central regulator of biological systems. While its primary sequence can encode protein, RNA can also fold into non-protein coding structural motifs that perform catalysis [1], bind small molecules [2], or serve as protein scaffolds [3]. Non-coding RNAs can conditionally govern gene expression [4] and have impressive regulatory capacity; small non-coding RNAs may modulate the expression of > 60% of human coding genes [5]. Built upon the growing number of well-characterized regulatory RNAs, novel RNA-based control systems are now being applied in microbial ([6], [7]) and mammalian biotechnology. Gene networks have been programmed to recognize and respond to cancer-associated miRNA profiles [8], shRNA-based genetic switches may support gene-therapy applications [9], and drug-responsive RNA sensors have been developed for T-cell therapy [10].

Despite the remarkable progress in characterizing RNA-based regulation and the promise of RNA biotechnology, the vast majority of RNA transcribed by the human genome remains functionally uncharacterized biological "Dark Matter." The pervasiveness of eukaryotic transcription came to light through numerous studies in the wake human genome project. Approximately 90% of the human genome is transcribed, yet only ~1.5% encodes for protein [11]. Increasingly sensitive sequencing technology has been used to catalog the human transcriptome, leading to the identification of many "long" non-coding RNAs (lncRNAs), which are distinguished from short regulatory RNA pathways by a length cut-off of greater than 200 base pairs. Recent studies have classified > 8000 intergenic lncRNAs [12],

which are often spliced, polyadenylated, and transcribed by RNA polymerase II in a highly tissue-specific manner. Some of these lncRNAs map to regions associated with disease by genome wide association studies (GWAS) [12] and the number of papers discussing lncRNA disease-associations has been growing each year [13].

Here we organize the rapidly expanding literature by discussing lncRNAs that exert epigenetic, transcriptional, and post-transcriptional control over gene expression. We provide examples of mammalian lncRNAs at each level of control by highlighting their putative regulatory mechanisms and interaction partners. We review the evidence linking these lncRNAs to diseases (**Figure 1**) and discuss new technologies that will improve understanding of lncRNA roles in human health.

Epigenetic control

Despite having identical genomes, different cell types exhibit unique and heritable gene expression patterns. Heritable variation ("-genetic") must be encoded in molecular signatures beyond ("-epi") DNA sequence itself [14]. These "epigenetic" signatures can be written to chromatin, the structural housing of genetic information in which DNA is wrapped around repeating octamers of histone proteins. Methylation of cytosine residues in DNA and post-translational histone modifications can specify the state of chromatin, resulting in transcriptional activation or silencing of the underlying DNA. In mammalian systems, the chromatin-remodeling machinery that write and erase these epigenetic signatures generally lack domains to specify DNA localization [15] and are thus dependent upon ancillary factors for their targeting to chromatin. Recent evidence suggests that lncRNAs encode this specificity by serving as scaffolds that tether chromatin-remodeling machinery to specific regions of the genome ((reviewed by [16]). Considering that epigenetic signatures must constrain gene expression patterns throughout development and are often dysregylated in diseases such as cancer [17], lncRNAs have garnered considerable attention for their role in epigenetic regulation of important loci, including tumor suppressor and developmental gene clusters.

The INK4b-ARF-INK4a tumor suppressor locus highlights the importance of lncRNAs in both epigenetic regulation and disease. This locus encodes three tumor suppressors (p15, p16, and ARF) [18] and is altered in ~30-40% of human tumors [19]. The lncRNA ANRIL (antisense non-coding RNA of the INK4 locus) participates in transcriptional repression of this locus through recruitment of two chromatin-remodeling complexes – Polycomb repressive complex 1 (PRC-1) [20] and PRC-2 [21] – that modify histones with signals for heterochromatin formation (e.g., trimethylation of histone 3 lysine 27 or "H3K27me3") and transcriptional silencing. Common disease genome-wide association studies (GWAS) have identified ANRIL as a susceptibility locus for numerous pathologies, including cancers, cardiovascular disease, and type II diabetes [22]. Genetic aberrations may contribute to disease through ANRIL dysregulation, as ANRIL over-expression is observed in cancers, such as leukemia [23], and SNPs can alter ANRIL splicing [24]. Providing additional support for lncRNA-mediated regulation of this locus, the lncRNA HEIH (High

Expression In Hepatocellular Carcinoma) participates in repression of p15 and p16 genes through its association with PRC-2 [25]. Further studies on these lncRNAs, as well as enhancer elements [26], should provide additional insights into the complex regulatory interactions that govern this disease-associated locus.

In addition tumor suppressor loci, developmental gene clusters are well-studied targets of lncRNA-mediated epigenetic activation and repression. Encoding a family of homeotic transcription factors critical for developmental patterning, HOX genes are an important example of activation by lncRNAs. Activation of HOX genes is correlated with H3K4 methylation, an epigenetic signal written by the lineage leukemia-1 (MLL-1) chromatin remodeling complex [27]. LncRNA-mediated targeting of MLL-1 for transcriptional activation of specific HOXA genes was first demonstrated by studies on the lncRNA HOTTIP (HOXA transcript at the distal tip) [28]. Chromosomal looping brings HOTTIP into close proximity with the 5' (distal) end of the locus, where HOTTIP recruits MLL-1 (by binding to its adapter protein WDR5) for transcriptional activation of distal HOXA genes. Further highlighting MLL-1 recruitment for HOXA gene activation, the lncRNA minstral (MIRA) directly binds MLL-1 and activates expression of HOXA6 and HOXA7 [29]. Future work may focus on the role of these lncRNAs in HOX-related developmental abnormalities [30], considering that HOTTIP knock-down is associated with distal forelimb shortening. In addition, it will be interesting to explore whether lncRNAs can recruit MLL-1 in cancers known to have aberrant activate chromatin domains [31].

Though epigenetic activation is a rather new paradigm, lncRNAs have long been associated with epigenetic silencing of developmental genes, including the HOX cluster. The lncRNA HOTAIR is expressed from the HOXC locus, but participates in transcriptional repression of HOXD loci [32] through recruitment of PRC-2 and LSD1 chromatin-remodeling complexes in *trans* [33]. The recruitment of these complexes simultaneously signals for heterochromatin formation (H3K27me3 via PRC-2) and removes H3K4me2 (via LSD1), a histone modification associated with transcriptional activation. Because it acts in *trans*, HOTAIR can target these complexes to different genomic regions [34], which is particularly important in the context of disease. The specificity of targeting is perturbed in breast cancer metastases, where HOTAIR is up-regulated ~ 100-fold and re-programs PRC2 localization so as to promote cell motility and matrix invasion, which are hallmarks of metastasis [35]. Paralleling its role in beast cancer metastasis, HOTAIR is also up-regulated, re-targets PRC2, and promotes metastasis in colorectal cancers [36]. Moreover, a deeper mechanistic understanding of lncRNA-mediated programming of oncogenic chromatin states may lead to novel strategies in cancer therapy.

LncRNA-mediated epigenetic silencing of developmental genes extends beyond the HOX cluster, and is particularly important for imprinting in sex-linked dosage compensation. A canonical example of lncRNA involvement in sex-linked gene dosage compensation is the process of X-chromosome inactivation, which equalizes expression of X-linked genes between sexes through silencing of one X-chromosome in female cells. Uniquely expressed from the inactive X-chromosome,

the lncRNA Xist binds PRC-2 through several stem-loops ([37], [38]) at its 5' end [39], leading to transcriptional silencing in *cis*. Though its regulatory network remains an intense area of investigation (reviewed by [40]), differential Xist expression levels can serve as markers for testicular and ovarian cancer outcomes ([41], [42]) and may play a role in autoimmune disorders [43]. Paralleling the role of Xist in X-chromosome inactivation, lncRNAs H19 and Kcnq1ot1 recruit repressive chromatin-remodeling complexes ([44], [45]) to an imprinted gene cluster on chromosome 11p15.5, which encodes cell growth regulatory factors. Down-regulation of H19 [46] and up-regulation of Kcnq1ot1 [47] are frequently observed Beckwith-Wiedemann syndrome, an over-growth disorder. H19 is also implicated in a number cancers, as it has been reported to serve as a tumor suppressor [48] .

Collectively, these examples demonstrate that lncRNAs help direct epigenetic signatures that constrain gene expression patterns in both development and disease. While these and other lncRNAs that template the epigenome remain very active targets of investigation, a growing number of lncRNAs have also been shown to exert regulatory control over transcriptional initiation at promoter DNA.

Transcriptional and co-transcriptional control

In addition to serving as scaffolds between chromatin and chromatin-remodeling machinery, lncRNAs serve as scaffolds that govern the activity and localization of transcription factors. Eukaryotic transcription is initiated through RNA polymerase II (polII) association with general transcription factors at promoter DNA, which give rise to the pre-initiation complex (PIC). Transcription factors can direct assembly of the PIC and may themselves be modulated by ligands or co-regulators (including co-activators or co-repressors) [49]. LncRNAs serve as co-regulators in several disease-related transcription factor signaling pathways, including the p53 response and several nuclear-receptor (NR) pathways.

The p53 transcription factor signaling network is a canonical mode of tumor suppression in which several lncRNAs serve as co-regulators [50]. The lincRNA p21 is up-regulated by p53 and co-regulates repression of > 1000 target genes by binding the hnRNP-K transcriptional repressor complex [51]. Paralleling the role of HOTAIR in PRC-2 localization, lincRNA-p21 targets hnRNP-K to repressed genes and is required for p53-induced apoptosis, warranting its classification as a tumor suppressor. Like lincRNA-p21, the lncRNA PANDA is also up-regulated by p53 in response to cell stress and protects the cell from apoptosis by repressing with NF-Y, a co-regulator of p53 that activates pro-apoptosis genes [52]. Further underscoring the importance of lncRNAs in the p53 signaling network, the lncRNA MEG3 (maternally expressed gene 3) enhances p53 binding to target gene promoters [53] and serves as a tumor suppressor, as its expression is down-regulation in numerous cancers (reviewed by [54]). Beyond cancer, GWAS have mapped SNPs to intron 6 of MEG3, associating this lncRNA with type 1 diabetes susceptibility [55].

Similar to their role in the p53 pathway, lncRNAs serve as co-regulators in nuclear receptor (NR) transcription factor signaling, which is important for proper development and dysregulated in diseases, such as cancer [56]. SRA (steroid receptor RNA activator) was the first lncRNA co-activator characterized [57] and its function requires a scaffold composed of six RNA stem-loops that may nucleate co-localization of proteins involved in transcriptional activation [58]. Follow-up work has shown that SRA interacts directly with over a dozen different proteins, targeting both positive (e.g., SRC-1, p68 and p72, Pus1p and Pus3p) as well as negative (e.g, Sharp and SLIRP) transcriptional regulators to promoters (reviewed by [59]). While SRA appears to exert regulatory control across multiple NR signaling pathways, the lncRNA growth-arrest specific 5 (Gas5) appears to specifically target the glucocortioid receptor (GR) though several hairpins that mimic the GR DNA binding site [60]. Moreover, Gas5 acts as a decoy [16], sensitizing cells to apoptosis by suppressing GR-signaling under low nutrient conditions. Underscoring the clinical importance of these lncRNAs, SRA is up-regulated in numerous cancers (reviewed by [61]) and Gas5 is down-regulated in breast cancer tissues, potentially providing a way for the cells to escape apoptosis during the process of oncogenesis [62].

Co-regulatory lncRNAs are involved in tumor suppression and oncogenesis beyond NR and p53 signaling pathways. Transcriptional regulation of the cell-cycle regulator cyclin D1 (CCND1) is governed by a set of lncRNAs that are up-regulated in response to heat shock or DNA damage. These lncRNAs associate with chromatin and TLS (translocated in liposarcoma) protein, simultaneously targeting TLS to the CCND1 promoter and allosterically modifying its C-terminus. Though the C-terminus of TLS normally represses activity of the N- terminus, lncRNA-binding relieves this internal inhibition, allowing the N-terminus to repress co-activators (CBP and p300) of histone acetyltransferase CREB and silence the CCND1 gene [63]. Because CCND1 is over-expressed in a variety of tumors [64], these lncRNAs may serve as tumor suppressors. In contrast, the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1) drives the proto-oncogene GAGE6 by repressing its transcriptional repressor, the hPSF tumor suppressor protein [65]. High MALAT-1 expression is associated with numerous cancers [66], including poor prognosis in lung cancer [67]. MALAT-1 also controls phospohrylation and localization of SR proteins, which dictate splicing patterns for many pre-RNA [68].

In addition to serving as transcriptional co-regulators, lncRNAs can also block PolII and general transcription factors (TFs) from interacting with promoter DNA and forming the pre-initiation complex (PIC). A well-studied example of PIC occlusion are Alu lncRNAs, which are expressed from prominent Alu repeats in the human genome [69]. Up-regulated under heat shock [70] and in cancers [71], Alu lncRNAs can bind to polII and block its association with promoter DNA [72]. Highlighting the role of Alu in disease, a recent study showed that accumulation of Alu lncRNAs in retinal cells leads to cytotoxicity and macular degeneration when Dicer1 is down-regulated [73]. Utilizing a different mechanism to inhibit PIC formation, lncRNAs expressed from the minor promoter of the human dihydrofolate reductase (DHFR) [74] gene can also repress PIC formation by establishing a

RNA:DNA triple helix at the major promoter [75], which blocks binding of general TFs. Because it is required for thymine biosynthesis in rapidly dividing cells, DHFR is the major target of cancer drug Methotrexate, which is used to treat childhood acute lymphoblastic leukemia (ALL); polymorphisms in the region encoding the DHFR lncRNA have been associated with poor outcomes in childhood ALL, potentially due to loss of repression over DHFR expression [76].

These examples show that lncRNA can serve as scaffolds that co-regulate transcription initiation and can repress transcription through direct interaction with polII or promoter DNA. Just as efforts have begun to explore the principles of lncRNA scaffold targeting in epigenetic regulation, it will be particularly interesting to understand how co-regulatory lncRNAs, such lincRNA-p21, can target specific promoters in transcription factor signaling networks. Beyond epigenetic and transcriptional control, lncRNAs also participate in post-transcriptional regulatory networks through their direct interaction with mRNAs and miRNAs.

Post-transcriptional control

Small regulatory RNAs post-transcriptionally modulate the expression of thousands of human genes and participate in signaling networks [77] that are dysregulated in disease [78]. Several recent studies have shown that lncRNAs are enmeshed in miRNA signaling networks by serving miRNA "sponges", which bind to and titrate the abundance of miRNAs available to bind their bona fide target transcripts [79]. Highlighting the role of these "competing endogenous RNAs" (ceRNAs) in development, the lncRNA MD1 binds two miRNAs that modulate the expression of developmental transcription factors in muscle cells [80] and is strongly down-regulated in Duchenne Muscular Dystrophy. In contrast, other ceRNAs are up-regulated in disease, such as "highly up-regulated in hepatocellular carcinoma" (HULC). This lncRNA drives its own expression by sequestering miR-372, a repressor of PRKACB kinase [81]. Like HULC, the pseudogene lncRNA PTEN-P1 has a role in cancer by acting as a sponge for miRNAs that modulate expression of the tumor suppressor phosphatase and tensin homolog (PTEN). PTEN-P1 may act as a tumor suppressor, as copy number losses of PTEN-P1 are observed in cancer [82] and cancer susceptibility is driven by subtle changes in PTEN dosage [83].

In addition to titration of miRNA abundance, lncRNAs post-transcriptionally modulate the stability of target transcripts through direct hybridization. The lncRNA BACE1-AS (BACE1 antisense) modulates expression of BACE1, an enzyme that cleaves amyloid precursor protein (APP) into amyloid-beta, a peptide that has been implicated in numerous neurological disorders. In Alzheimer's disease (AD), BACE1-AS is up-regulated and stabilizes the BACE-1 mRNA [84], potentially through miRNA binding site occlusion [85]. Elevated BACE1 levels result in pathogenic accumulation of amyloid-beta peptide, which further drives BACE1-AS expression. Moreover, BACE1-AS may serve as a biomarker for early detection of AD and siRNAs targeting BACE1 is a potential strategy for Alzheimer's treatment [86].

Future perspectives

Collectively, these examples provide a compelling but incomplete view of lncRNA regulation and involvement in pathogenesis. Sequencing technology continues to improve exponentially [87], driving discovery of thousands of lncRNAs that are up-regulated in diseases such as prostate [88], liver [89], and hepatocellular [25] cancers. Though our ability to identify lncRNAs that correlate with disease far outpaces our ability to understand the mechanistic link, sequencing technologies also provide several ways to help close this gap. Because lncRNAs exert regulatory function through their interactions with other molecules, numerous sequencing based technologies have been developed for high-throughput mapping of the lncRNA interactome. RNA immunoprecipitation followed by sequencing (RIP-seq) identified thousands of *cis* and *trans*-acting lncRNAs that associate with PRC-2 [90] and direct cross-linking of RNA-protein interactions *in vivo* is a promising strategy to identify direct interactions [91]. Whereas these methods take a protein-centric view, chromatin isolation by RNA purification (ChIRP) takes an RNA-centric view. Using ChIRP, an RNA can be isolated from a cross-linked pool of chromatin to retrieve and enumerate associated DNA sequences and protein [34]. Emerging technologies for transcriptome-wide determination of RNA structure provide complimentary information [92] (**Figure 2a**), allowing researchers to associate interaction domains with the underlying structures that may encode function.

Just as advances in DNA sequencing have lowered the barrier to acquisition of large-scale observational data, advances in DNA synthesis will increase the scale of perturbations that researchers can make, providing powerful ways to test hypotheses generated from the abovementioned profiling methods. Programmable arrays of synthetic RNA molecules and high-throughput assays for binding affinity [93] may provide detailed biophysical maps of the lncRNA interactome *in vitro*. *In vivo* studies will benefit from synthetic shRNA libraries, enabling high-throughput loss of function profiling [94]. Gene synthesis technologies [87] should enable construction of synthetic lncRNAs for identifying minimal functional domains ([3], [39]), exploring structural and functional modularity ([70], [39], [95]), and testing structure-function relationships [96] (**Figure 2b**). Using the power of DNA synthesis to explore lncRNA functional composition is a major opportunity for the field [97].

Ongoing efforts to identify lncRNAs, quantitatively map their interactome, and understand their functional composition have at least three important clinical implications. First, lncRNAs serve as bio-markers for diseases including breast cancer [35], hepatocellular cancer ([98], [25]), liver cancer [99], prostate cancer [88], lung cancer [67], and Alzheimer's Disease [100]. Contributing to their potential utility as disease biomarkers, some lncRNAs are detectable in body fluids [101]. Second, understanding lncRNA functional composition should make it possible to predict the effect of mutations [102], just as knowledge of the genetic code now makes it possible to predict the impact of mutations within protein coding regions [103]. Finally, well-characterized motifs and rules for their composition may enable design of therapeutic lncRNAs for control over nuclear organization or the

epigenome [104]. Considering that well-studied lncRNAs regulate development of diverse tissues [105], can target specific regions of DNA [106], and associate with chromatin-remodeling machinery [38], synthetic lncRNAs may recombine this existing regulatory diversity in novel ways. Moreover, progress in lncRNA science should benefit the bourgeoning field of RNA biotechnology [107], resulting in new strategies for disease amelioration or regenerative medicine applications [108].

Acknowledgements
We thank Robert Spitale for helpful comments on the manuscript. We thank Pedro Batista and Ci Chu for feedback on the figures. We apologize to colleagues whose work could not be discussed and cited due to space limitations. Supported by National Defense Science and Engineering Graduate Fellowship (L.M.), and by California Institute for Regenerative Medicine, National Institutes of Health (H.Y.C.). H.Y.C. is an Early Career Scientist of the Howard Hughes Medical Institute.

Figure legends

Figure 1
Functions of known disease-linked lncRNAs

Figure 2
A) Differential expression profiling has been used to discover lncRNAs that are up-regulated in specific tissues or diseases. Comparative genomics can be used to infer functional domains within these lncRNAs based upon conservation of RNA sequence or structure. Technologies provide unique windows into the features of lncRNAs, which are represented as amorphous gray bars. PARS, ChIRP, and CLIP-seq enable rapid enumeration of lncRNA structure, genomic binding sites, and protein partners, respectively. B) Perturbing lncRNA structure and organization is a powerful way to test hypotheses generated from high-throughput observational datasets. Directed deletion of lncRNA domains can identify the minimal sequence and structural motifs that are necessary for function. Compensatory mutations in binding motifs can be used to test whether structure, rather than sequence, is sufficient for function. Chimeric lncRNAs can explore motif modularity.

References

1. Cech TR: **Crawling Out of the RNA World**. *Cell* 2009, **136**:599–602.

2. Caprara MG, Nilsen TW: **RNA: versatility in form and function.** *Nat. Struct. Biol.* 2000, **7**:831–833.

3. Zappulla DC, Cech TR: **RNA as a flexible scaffold for proteins: yeast telomerase and beyond.** *Cold Spring Harb. Symp. Quant. Biol.* 2006, **71**:217–224.

4. Mandal M, Breaker RR: **Gene regulation by riboswitches.** *Nat Rev Mol Cell Biol* 2004, **5**:451–463.

5. Friedman RC, Farh KK-H, Burge CB, Bartel DP: **Most mammalian mRNAs are conserved targets of microRNAs.** *Genome Res.* 2009, **19**:92–105.

6. Delebecque CJ, Lindner AB, Silver PA, Aldaye FA: **Organization of Intracellular Reactions with Rationally Designed RNA Assemblies**. *Science* 2011, **333**:470–474.

7. Sinha J, Reyes SJ, Gallivan JP: **Reprogramming bacteria to seek and destroy an herbicide.** *Nat. Chem. Biol.* 2010, **6**:464–470.

8. Xie Z, Wroblewska L, Prochazka L, Weiss R, Benenson Y: **Multi-Input RNAi-Based Logic Circuit for Identification of Specific Cancer Cells**. *Science* 2011, **333**:1307–1311.

9. Deans TL, Cantor CR, Collins JJ: **A tunable genetic switch based on RNAi and repressor proteins for regulating gene expression in mammalian cells.** *Cell* 2007, **130**:363–372.

10. Chen YY, Jensen MC, Smolke CD: **Genetic control of mammalian T-cell proliferation with synthetic RNA regulatory systems.** *Proc. Natl. Acad. Sci. U.S.A.* 2010, **107**:8531–8536.

11. Lander ES: **Initial impact of the sequencing of the human genome**. *Nature* 2011, **470**:187–197.

12. Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A, Rinn JL: **Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses.** *Genes & Development* 2011, doi:10.1101/gad.17446611.

13. Gibb EA, Brown CJ, Lam WL: **The functional role of long non-coding RNA in human carcinomas**. *Molecular Cancer* 2011, **10**:38.

14. Bonasio R, Tu S, Reinberg D: **Molecular Signals of Epigenetic States**. *Science* 2010, **330**:612–616.

15. Margueron R, Reinberg D: **The Polycomb complex PRC2 and its mark in life.** *Nature* 2011, **469**:343–349.

16. Wang KC, Chang HY: **Molecular Mechanisms of Long Noncoding RNAs.** *Molecular Cell* 2011, **43**:904–914.

17. Bracken AP, Helin K: **Polycomb group proteins: navigators of lineage pathways led astray in cancer**. *Nat Rev Cancer* 2009, **9**:773–784.

18. Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M: **The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus.** *Nature* 1999, **397**:164–168.

19. Kim WY, Sharpless NE: **The regulation of INK4/ARF in cancer and aging.** *Cell* 2006, **127**:265–275.

20. Yap KL, Li S, Muñoz-Cabello AM, Raguz S, Zeng L, Mujtaba S, Gil J, Walsh MJ, Zhou M-M: **Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a.** *Molecular Cell* 2010, **38**:662–674.

21. Kotake Y, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M, Xiong Y: **Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15INK4B tumor suppressor gene**. *Oncogene* 2010, **30**:1956–1962.

22. Pasmant E, Sabbagh A, Vidaud M, Bieche I: **ANRIL, a long, noncoding RNA, is an unexpected major hotspot in GWAS**. *The FASEB Journal* 2011, **25**:444–448.

23. Yu W, Gius D, Onyango P, Muldoon-Jacobs K, Karp J, Feinberg AP, Cui H: **Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA.** *Nature* 2008, **451**:202–206.

24. Burd CE, Jeck WR, Liu Y, Sanoff HK, Wang Z, Sharpless NE: **Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk.** *PLoS Genet* 2010, **6**:e1001233.

25. Yang F, Zhang L, Huo X-S, Yuan J-H, Xu D, Yuan S-X, Zhu N, Zhou W-P, Yang G-S, Wang Y-Z, et al.: **Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans.** *Hepatology* 2011, **54**:1679–1689.

26. Harismendy O, Notani D, Song X, Rahim NG, Tanasa B, Heintzman N, Ren B, Fu X-D, Topol EJ, Rosenfeld MG, et al.: **9p21 DNA variants associated with coronary artery disease impair interferon-γ signalling response.** *Nature* 2011, **470**:264–268.

27. Ruthenburg AJ, Allis CD, Wysocka J: **Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark.** *Molecular Cell* 2007, **25**:15–30.

28. Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, Lajoie BR, Protacio A, Flynn RA, Gupta RA, et al.: **A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression.** *Nature* 2011, **472**:120–124.

29. Bertani S, Sauer S, Bolotin E, Sauer F: **The Noncoding RNA Mistral Activates Hoxa6 and Hoxa7 Expression and Stem Cell Differentiation by Recruiting MLL1 to Chromatin**. *Molecular Cell* 2011, **43**:1040–1046.

30. Shah N, Sukumar S: **The Hox genes and their roles in oncogenesis.** *Nat Rev Cancer* 2010, **10**:361–371.

31. Aiden AP, Rivera MN, Rheinbay E, Ku M, Coffman EJ, Truong TT, Vargas SO, Lander ES, Haber DA, Bernstein BE: **Wilms tumor chromatin profiles highlight stem cell properties and a renal developmental network.** *Cell Stem Cell* 2010, **6**:591–602.

32. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E, et al.: **Functional Demarcation of Active and Silent Chromatin Domains in Human HOX Loci by Noncoding RNAs**. *Cell* 2007, **129**:1311–1323.

33. Tsai M-C, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY: **Long noncoding RNA as modular scaffold of histone modification complexes.** *Science* 2010, **329**:689–693.

34. Chu C, Qu K, Zhong FL, Artandi SE, Chang HY: **Genomic Maps of Long Noncoding RNA Occupancy Reveal Principles of RNA-Chromatin Interactions.** *Molecular Cell* 2011, doi:10.1016/j.molcel.2011.08.027.

35. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai M-C, Hung T, Argani P, Rinn JL, et al.: **Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis.** *Nature* 2010, **464**:1071–1076.

36. Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, et al.: **Long non-coding RNA HOTAIR regulates Polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers**. *Cancer Research* 2011, doi:10.1158/0008-5472.CAN-11-1021.

37. Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT: **Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome.** *Science* 2008, **322**:750–756.

38. Maenner S, Blaud M, Fouillen L, Savoye A, Marchand V, Dubois A, Sanglier-Cianférani S, Van Dorsselaer A, Clerc P, Avner P, et al.: **2-D structure of the A region of Xist RNA and its implication for PRC2 association.** *PLoS Biol.* 2010, **8**:e1000276.

39. Wutz A, Rasmussen TP, Jaenisch R: **Chromosomal silencing and localization are mediated by different domains of Xist RNA.** *Nature*

Genetics 2002, **30**:167–174.

40. Augui S, Nora EP, Heard E: **Regulation of X-chromosome inactivation by the X-inactivation centre.** *Nature Publishing Group* 2011, **12**:429–442.

41. Kawakami T, Okamoto K, Ogawa O: **ScienceDirect - The Lancet : XISTunmethylated DNA fragments in male-derived plasma as a tumour marker for testicular cancer**. *The Lancet* 2004, [no volume].

42. Huang K-C, Rao PH, Lau CC, Heard E, Ng S-K, Brown C, Mok SC, Berkowitz RS, Ng S-W: **Relationship of XIST expression and responses of ovarian cancer to chemotherapy.** *Mol. Cancer Ther.* 2002, **1**:769–776.

43. Broen JCA, Wolvers-Tettero ILM, Geurts-van Bon L, Vonk MC, Coenen MJH, Lafyatis R, Radstake TRDJ, Langerak AW: **Skewed X chromosomal inactivation impacts T regulatory cell function in systemic sclerosis.** *Ann. Rheum. Dis.* 2010, **69**:2213–2216.

44. Zhao J, Ohsumi TK, Kung JT, Ogawa Y, Grau DJ, Sarma K, Song JJ, Kingston RE, Borowsky M, Lee JT: **Genome-wide identification of polycomb-associated RNAs by RIP-seq.** *Molecular Cell* 2010, **40**:939–953.

45. Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, Nagano T, Mancini-Dinardo D, Kanduri C: **Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation.** *Molecular Cell* 2008, **32**:232–246.

46. Gabory A, Jammes H, Dandolo L: **The H19 locus: role of an imprinted non-coding RNA in growth and development.** *Bioessays* 2010, **32**:473–480.

47. Chiesa N, De Crescenzo A, Mishra K, Perone L, Carella M, Palumbo O, Mussa A, Sparago A, Cerrato F, Russo S, et al.: **The KCNQ1OT1 Imprinting Control Region and non-coding RNA: new properties derived from the study of Beckwith-Wiedemann syndrome and Silver-Russell syndrome cases.** *Human Molecular Genetics* 2011, doi:10.1093/hmg/ddr419.

48. Yoshimizu T, Miroglio A, Ripoche M-A, Gabory A, Vernucci M, Riccio A, Colnot S, Godard C, Terris B, Jammes H, et al.: **The H19 locus acts in vivo as a tumor suppressor.** *Proc. Natl. Acad. Sci. U.S.A.* 2008, **105**:12417–12422.

49. Perissi V, Jepsen K, Glass CK, Rosenfeld MG: **Deconstructing repression: evolving models of co-repressor action**. *Nature Publishing Group* 2010, **11**:109–123.

50. Rinn JL, Huarte M: **To repress or not to repress: This is the guardian's question**. *Trends in Cell Biology* 2011, **21**:344–353.

51. Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M, et al.: **A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response.** *Cell* 2010, **142**:409–419.

52. Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, Horlings HM, Shah N, Umbricht C, Wang P, et al.: **Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters**. *Nature Genetics* 2011, **43**:621–629.

53. Zhou Y, Zhong Y, Wang Y, Zhang X, Batista DL, Gejman R, Ansell PJ, Zhao J, Weng C, Klibanski A: **Activation of p53 by MEG3 non-coding RNA.** *J. Biol. Chem.* 2007, **282**:24731–24742.

54. Benetatos L, Vartholomatos G, Hatzimichael E: **MEG3 imprinted gene contribution in tumorigenesis.** *Int. J. Cancer* 2011, **129**:773–779.

55. Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Todd JA, Clayton DG: **The imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes.** *Nature Genetics* 2010, **42**:68–71.

56. Gronemeyer H, Gustafsson J-A, Laudet V: **Principles for modulation of the nuclear receptor superfamily.** *Nat Rev Drug Discov* 2004, **3**:950–964.

57. Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, Tsai SY, Tsai MJ, O'Malley BW: **A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex.** *Cell* 1999, **97**:17–27.

58. Lanz RB, Razani B, Goldberg AD, O'Malley BW: **Distinct RNA motifs are important for coactivation of steroid hormone receptors by steroid receptor RNA activator (SRA).** *Proc. Natl. Acad. Sci. U.S.A.* 2002, **99**:16081–16086.

59. Colley SM, Leedman PJ: **Steroid Receptor RNA Activator - A nuclear receptor coregulator with multiple partners: Insights and challenges.** *Biochimie* 2011, doi:10.1016/j.biochi.2011.07.004.

60. Kino T, Hurt DE, Ichijo T, Nader N, Chrousos GP: **Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor.** *Sci Signal* 2010, **3**:ra8.

61. Leygue E: **Steroid receptor RNA activator (SRA1): unusual bifaceted gene products with suspected relevance to breast cancer.** *Nucl Recept Signal* 2007, **5**:e006.

62. Mourtada-Maarabouni M, Pickard MR, Hedge VL, Farzaneh F, Williams GT: **GAS5, a non-protein-coding RNA, controls apoptosis and is**

downregulated in breast cancer. *Oncogene* 2009, **28**:195–208.

63. Wang X, Arai S, Song X, Reichart D, Du K, Pascual G, Tempst P, Rosenfeld MG, Glass CK, Kurokawa R: **Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription.** *Nature* 2008, **454**:126–130.

64. Diehl JA: **Cycling to cancer with cyclin D1.** *Cancer Biol. Ther.* 2002, **1**:226–231.

65. Li L, Feng T, Lian Y, Zhang G, Garen A, Song X: **Role of human noncoding RNAs in the control of tumorigenesis.** *Proc. Natl. Acad. Sci. U.S.A.* 2009, **106**:12956–12961.

66. Guffanti A, Iacono M, Pelucchi P, Kim N, Soldà G, Croft LJ, Taft RJ, Rizzi E, Askarian-Amiri M, Bonnal RJ, et al.: **A transcriptional sketch of a primary human breast cancer by 454 deep sequencing.** *BMC Genomics* 2009, **10**:163.

67. Ji P, Diederichs S, Wang W, Boing S, Metzger R, Schneider P, Tidow N, Brandt B, Buerger H, Bulk E, et al.: **MALAT-1, a novel noncoding RNA, and thymosin beta 4 predict metastasis and survival in early-stage non-small cell lung cancer**. *Oncogene* 2003, **22**:8031–8041.

68. Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, Freier SM, Bennett CF, Sharma A, Bubulya PA, et al.: **The Nuclear-Retained Noncoding RNA MALAT1 Regulates Alternative Splicing by Modulating SR Splicing Factor Phosphorylation**. *Molecular Cell* 2010, **39**:925–938.

69. Batzer MA, Deininger PL: **Alu repeats and human genomic diversity.** *Nat. Rev. Genet.* 2002, **3**:370–379.

70. Mariner PD, Walters RD, Espinoza CA, Drullinger LF, Wagner SD, Kugel JF, Goodrich JA: **Human Alu RNA is a modular transacting repressor of mRNA transcription during heat shock.** *Molecular Cell* 2008, **29**:499–509.

71. Tang R-B, Wang H-Y, Lu H-Y, Xiong J, Li H-H, Qiu X-H, Liu H-Q: **Increased level of polymerase III transcribed Alu RNA in hepatocellular carcinoma tissue.** *Mol. Carcinog.* 2005, **42**:93–96.

72. Yakovchuk P, Goodrich JA, Kugel JF: **B2 RNA and Alu RNA repress transcription by disrupting contacts between RNA polymerase II and promoter DNA within assembled complexes.** *Proc. Natl. Acad. Sci. U.S.A.* 2009, **106**:5569–5574.

73. Kaneko H, Dridi S, Tarallo V, Gelfand BD, Fowler BJ, Cho WG, Kleinman ME, Ponicsan SL, Hauswirth WW, Chiodo VA, et al.: **DICER1 deficit induces Alu**

RNA toxicity in age-related macular degeneration. *Nature* 2011, **471**:325–.

74. Martianov I, Ramadass A, Serra Barros A, Chow N, Akoulitchev A: **Repression of the human dihydrofolate reductase gene by a non-coding interfering transcript.** *Nature* 2007, **445**:666–670.

75. Blume SW, Meng Z, Shrestha K, Snyder RC, Emanuel PD: **The 5'-untranslated RNA of the human dhfr minor transcript alters transcription pre-initiation complex assembly at the major (core) promoter.** *J. Cell. Biochem.* 2003, **88**:165–180.

76. Al-Shakfa F, Dulucq S, Brukner I, Milacic I, Ansari M, Beaulieu P, Moghrabi A, Laverdière C, Sallan SE, Silverman LB, et al.: **DNA variants in region for noncoding interfering transcript of dihydrofolate reductase gene and outcome in childhood acute lymphoblastic leukemia.** *Clin. Cancer Res.* 2009, **15**:6931–6938.

77. Inui M, Martello G, Piccolo S: **MicroRNA control of signal transduction**. *Nat Rev Mol Cell Biol* 2010, **11**:264–275.

78. Esquela-Kerscher A, Slack FJ: **Oncomirs — microRNAs with a role in cancer**. *Nat Rev Cancer* 2006, **6**:259–269.

79. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP: **A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?** *Cell* 2011, **146**:353–358.

80. Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A, Bozzoni I: **A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA.** *Cell* 2011, **147**:358–369.

81. Wang J, Liu X, Wu H, Ni P, Gu Z, Qiao Y, Chen N, Sun F, Fan Q: **CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer.** *Nucleic Acids Research* 2010, **38**:5366–5383.

82. Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, Pandolfi PP: **A coding-independent function of gene and pseudogene mRNAs regulates tumour biology.** *Nature* 2010, **465**:1033–1038.

83. Alimonti A, Carracedo A, Clohessy JG, Trotman LC, Nardella C, Egia A, Salmena L, Sampieri K, Haveman WJ, Brogi E, et al.: **Subtle variations in Pten dose determine cancer susceptibility.** *Nature Genetics* 2010, **42**:454–458.

84. Faghihi MA, Modarresi F, Khalil AM, Wood DE, Sahagan BG, Morgan TE,

Finch CE, St Laurent G, Kenny PJ, Wahlestedt C: **Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase.** *Nat. Med.* 2008, **14**:723–730.

85. Faghihi MA, Zhang M, Huang J, Modarresi F, Van der Brug MP, Nalls MA, Cookson MR, St Laurent G, Wahlestedt C: **Evidence for natural antisense transcript-mediated inhibition of microRNA function.** *Genome Biol* 2010, **11**:R56.

86. Modarresi F, Faghihi MA, Patel NS, Sahagan BG, Wahlestedt C, Lopez-Toledano MA: **Knockdown of BACE1-AS Nonprotein-Coding Transcript Modulates Beta-Amyloid-Related Hippocampal Neurogenesis.** *Int J Alzheimers Dis* 2011, **2011**:929042.

87. Carr PA, Church GM: **Genome engineering.** *Nature Biotechnology* 2009, **27**:1151–1162.

88. Prensner JR, Iyer MK, Balbin OA, Dhanasekaran SM, Cao Q, Brenner JC, Laxman B, Asangani IA, Grasso CS, Kominsky HD, et al.: **Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression**. *Nature Biotechnology* 2011, **29**:744–758.

89. Panzitt K, Tschernatsch MMO, Guelly C, Moustafa T, Stradner M, Strohmaier HM, Buck CR, Denk H, Schroeder R, Trauner M, et al.: **Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA.** *Gastroenterology* 2007, **132**:330–342.

90. Zhao J, Ohsumi TK, Kung JT, Ogawa Y, Grau DJ, Sarma K, Song JJ, Kingston RE, Borowsky M, Lee JT: **Genome-wide Identification of Polycomb-Associated RNAs by RIP-seq**. *Molecular Cell* 2010, **40**:939–953.

91. Chi SW, Zang JB, Mele A, Darnell RB: **Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps**. *Nature* 2009, **460**:479–486.

92. Wan Y, Kertesz M, Spitale RC, Segal E, Chang HY: **Understanding the transcriptome through RNA structure**. *Nature Publishing Group* 2011, **12**:641–655.

93. Maerkl SJ: **Next generation microfluidic platforms for high-throughput protein biochemistry**. *Current Opinion in Biotechnology* 2011, **22**:59–65.

94. Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, Young G, Lucas AB, Ach R, Bruhn L, et al.: [no date].

95. Mohammad F, Pandey RR, Nagano T, Chakalova L, Mondal T, Fraser P, Kanduri C: **Kcnq1ot1/Lit1 noncoding RNA mediates transcriptional**

silencing by targeting to the perinucleolar region. *Mol. Cell. Biol.* 2008, **28**:3713–3728.

96. Zhang X, Gejman R, Mahta A, Zhong Y, Rice KA, Zhou Y, Cheunsuchon P, Louis DN, Klibanski A: **Maternally expressed gene 3, an imprinted noncoding RNA gene, is associated with meningioma pathogenesis and progression.** *Cancer Research* 2010, **70**:2350–2358.

97. Elowitz M, Lim WA: **Build life to understand it.** *Nature* 2010, **468**:889–890.

98. Matouk IJ, DeGroot N, Mezan S, Ayesh S, Abu-Iail R, Hochberg A, Galun E: **The H19 Non-Coding RNA Is Essential for Human Tumor Growth**. *Plos One* 2007, **2**:–.

99. Yang Z, Zhou L, Wu L-M, Lai M-C, Xie H-Y, Zhang F, Zheng S-S: **Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation.** *Ann. Surg. Oncol.* 2011, **18**:1243–1250.

100. Coulson DTR, Beyer N, Quinn JG, Brockbank S, Hellemans J, Irvine GB, Ravid R, Johnston JA: **BACE1 mRNA expression in Alzheimer's disease postmortem brain tissue.** *J. Alzheimers Dis.* 2010, **22**:1111–1122.

101. de Kok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeney LA, Aalders TW, Swinkels DW, Schalken JA: **DD3(PCA3), a very sensitive and specific marker to detect prostate tumors.** *Cancer Research* 2002, **62**:2695–2698.

102. Wapinski O, Chang HY: **Long noncoding RNAs and human disease**. *Trends in Cell Biology* 2011, **21**:354–361.

103. Cooper GM, Shendure J: **Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data**. *Nature Publishing Group* 2011, **12**:628–640.

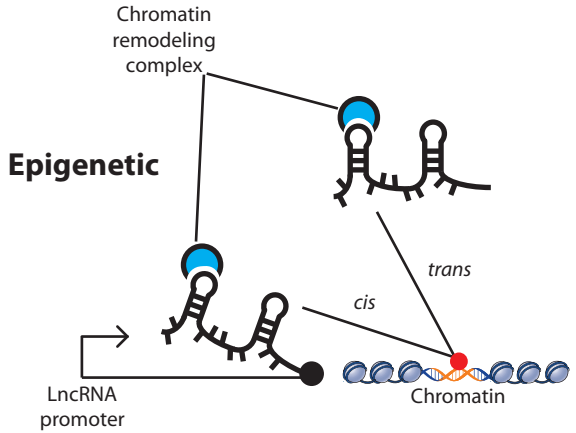
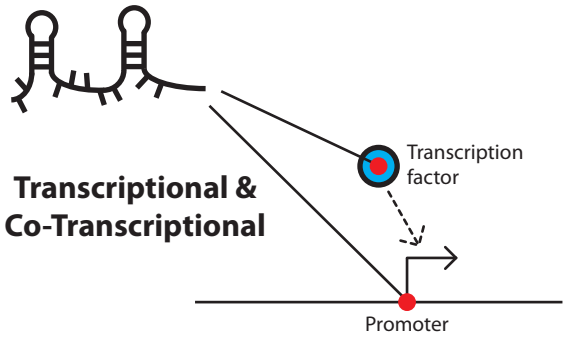
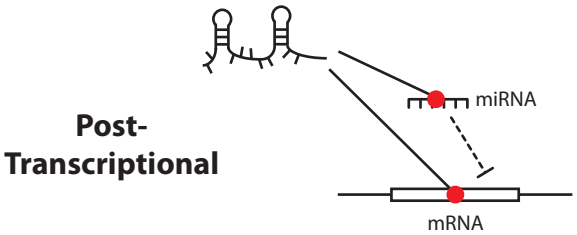
104. Bashor CJ, Horwitz AA, Peisajovich SG, Lim WA: **Rewiring Cells: Synthetic Biology as a Tool to Interrogate the Organizational Principles of Living Systems**. *Annu. Rev. Biophys.* 2010, **39**:515–537.

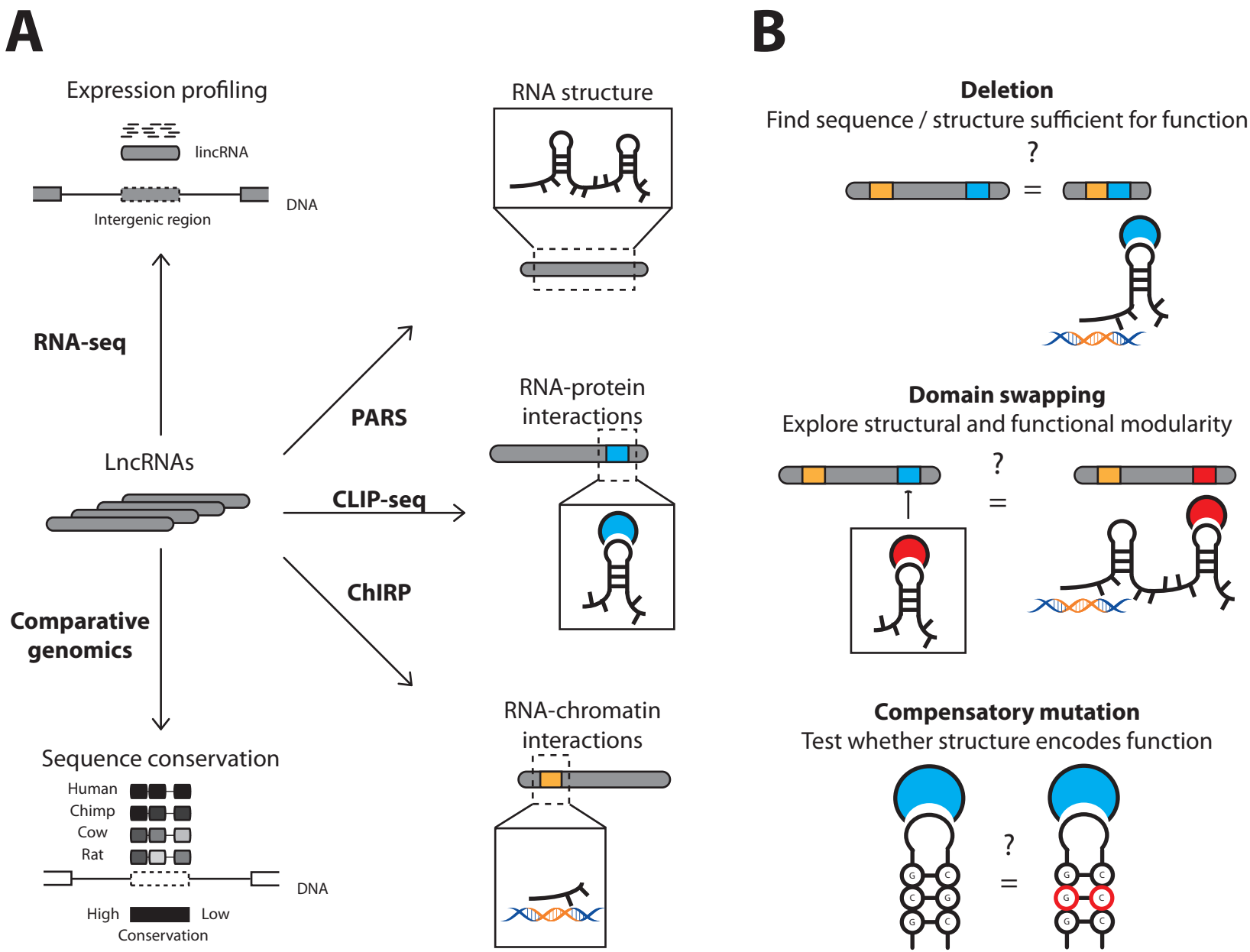
105. Bond AM, Vangompel MJW, Sametsky EA, Clark MF, Savage JC, Disterhoft JF, Kohtz JD: **Balanced gene regulation by an embryonic brain ncRNA is critical for adult hippocampal GABA circuitry.** *Nat. Neurosci.* 2009, **12**:1020–1027.

106. Schmitz K-M, Mayer C, Postepska A, Grummt I: **Interaction of noncoding RNA with the rDNA promoter mediates recruitment of DNMT3b and silencing of rRNA genes.** *Genes & Development* 2010, **24**:2264–2269.

107. Liu CC, Arkin AP: **The Case for RNA**. *Science* 2010, **330**:1185–1186.

108. Ruder WC, Lu T, Collins JJ: **Synthetic Biology Moving into the Clinic**. *Science* 2011, **333**:1248–1252.

General points of regulation	LncRNA	Interactions	Clinical relevance	References
	ANRIL	Binds PRC-1 and 2 ; Represses INK4b-ARF-INK4a tumor suppressor locus	Oncogenic ; SNPs associated with susceptibility to coronary disease, type 2 diabetes, and cancers	20-24
	H19	Potentially binds PRC-2 ; Represses imprinted genes in *trans* ; Generates miRNA	Tumor suppressor ; Down-regulated in Beckwith-Wiedemann syndrome, a growth disorder	44, 46, 48
	HEIH	Binds EZH2 sub-unit of PRC-2 ; Represses PRC2 target genes, including INK4b-ARF-INK4a locus	Oncogenic ; Bio-marker for hepatocellular carcinoma recurrence and post-operative survival	25
	HOTAIR	Binds LSD1 and PRC-2 ; Represses of HOXD and other genomic loci in *trans*	Oncogenic ; Over-expression mis-targets PRC2 Promotes breast / colorectal cancer metastasis	32-36
	HOTTIP	Binds WDR5 (adapter protein for MLL-1 complex) ; Activates distal HOXA genes	Down-regulation leads to shortening of distal forelimb bones ; Possible role in HOX disorders / leukemia	28
	Kcnq1ot1	Binds G9a and PRC-2 ; Represses imprinted genes at 11p15 locus	Up-regulated in Beckwith-Wiedemann syndrome	45, 47
	MIRA	Binds MLL-1 complex ; Activates HOXA6 and 7	TBD ; Possible role in HOX disorders / leukemia	29
	Xist	Binds PRC-2 and other proteins in X-chromosome inactivation (XCI) network ; Repressive	Skewed XCI in disease (e.g., autoimmune disorders) ; Marker for testicular and ovarian cancer outcomes	37-43
	Alu	Bind to PolII ; Represses transcription by blocked PolII-DNA interaction	Oncogenic ; Up-regulated in hepatocellular carinoma ; Accumulation drives macular degeneration	68-72
	CCND1	Binds TLS and chromatin ; Represses transcription of CCND1	Tumor suppressor ; CCND1 over-expressed in cancers	63-64
	DHFR	Binds promoter DNA via triple-helix ; Represses transcription by blocking PIC formation	Tumor suppressor ; Polymorpisms lead to increased DHFR expression / poor outcomes childhood leukemia	73-75
	Evf2	Binds transcription factors (DLX2 and MECP2)	Role in neuro-developmental disorders	105
	Gas5	Binds glucocortioid receptor (GR) ; Represses transcription of GR target genes	Tumor-suppressor ; Down-regulated in breast cancer	60, 62
	MALAT-1 (NEAT2)	Binds and represses hPSF, a tumor suppressor ; RNA scaffold for activation of E2F target genes	Oncogenic ; hPSF repressor ; Up-regulated in cancers (e.g., lung)	65-68
	MEG3	Binds p53 ; Activates p53 signaling and promotes growth suppression	Tumor suppressor ; Down-regulated in cancers ; SNPs increase susceptibility to type 1 diabetes	53-55
	lincRNA-p21	Binds hnRNPk, repressor complex in the p53 signaling pathway ; Targets hnRNPk to promoters	Tumor suppressor ; Mediates repression of > 1000 p53 target genes	51
	PANDA	Binds and represses NF-Y, an activator of pro-apoptotic genes in p53 pathway	Suppressor of p53-mediated apoptosis	52
	SRA	Binds steroid receptor and other NRs ; Co-regulates signaling in multiple NR pathways	Oncogenic ; Up-regulated and drives NR signaling in cancers (e.g., breast, prostate)	57-59
	BACE1-AS	Binds BACE1 mRNA ; Activates expression by stabilizing BACE1 transcript	Over-expressed in Alzheimer's disease (AD)	84-86
	HULC	Binds mi-372 ; Expression maintained by autoregulatory feedback	Highly-expressed in liver cancer	13, 81
	MD1	Binds miR-133 and miR-135, which modulate developmental transcription factors in muscle cells	TBD ; Down-regulated in Duchenne Muscular Dystrophy cells	80
	PTEN-P1	Binds miRNAs that modulate expression of PTEN tumor suppressor	Tumor suppressor ; Copy number losses in cancers, driving changes in PTEN tumor suppressor dosage	82, 83


A
Expression profiling
lincRNA
DNA
Intergenic region
RNA-seq
LncRNAs
Comparative genomics
Sequence conservation
Human
Chimp
Cow
Rat
DNA
High
Low
Conservation
PARS
CLIP-seq
ChIRP
RNA structure
RNA-protein interactions
RNA-chromatin interactions
B
Deletion
Find sequence / structure sufficient for function
?
=
Domain swapping
Explore structural and functional modularity
?
=
Compensatory mutation
Test whether structure encodes function
?
=
G
C