Long Noncoding RNAs: Cellular Address Codes in Development and Disease

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http://dx.doi.org/10.1016/j.cell.2013.02.012

In biology as in real estate, location is a cardinal organizational principle that dictates the accessibility and flow of informational traffic. An essential question in nuclear organization is the nature of the address code—how objects are placed and later searched for and retrieved. Long noncoding RNAs (lncRNAs) have emerged as key components of the address code, allowing protein complexes, genes, and chromosomes to be trafficked to appropriate locations and subject to proper activation and deactivation. lncRNA-based mechanisms control cell fates during development, and their dysregulation underlies some human disorders caused by chromosomal deletions and translocations.

Introduction

From a single cell to an entire organism, spatial positioning is a key problem in biology. It is well appreciated that robust systems sort and distribute macromolecules, a property essential for the function of cells and tissues (Shevtsov and Dundr, 2011; Wolpert, 2011). A historical example illustrates the general utility of spatial organization. As the Roman Empire expanded and the Romans were faced with the need to construct cities in new lands, they developed a city prototype that included a group of answers to the many practical problems related to the creation and maintenance of a city (Figure 1A). This was a universal plan of simple execution. City walls protected the citizens from attack and delimited the city. At the center stood the forum, where the business and political activities of the city were concentrated. Fountains were placed throughout the city to supply water, and other spaces, such as amphitheaters, temples, and baths, were dedicated to organize daily activities. Thus, a group of structures analogous in function was always present in an organization that follows the original prototype (Grimal and Woloch, 1983).

Just like the Roman city, the nucleus of the eukaryotic cell is a highly organized space (Figure 1B). Evolution gave rise to a "nuclear" prototype that provides answers to the many challenges the cell has to respond to maintain homeostasis and growth, though subject to developmental specialization (Solovei et al., 2009). Chromosomes are not randomly organized in the nucleus, and during interphase, each chromosome occupies a discrete territory (reviewed in Cremer and Cremer, 2010). Furthermore, whereas the densely compacted heterochromatin is localized at the nuclear envelope, euchromatin localizes to the interior regions of the nucleus. Gene expression is also localized and occurs mostly at nuclear center. In addition, active genes that are coregulated are often found forming clusters. During development, individual loci such as immunoglobulin or *Hox* genes are known to change position within the nucleus according to their transcriptional status (reviewed in Misteli, 2007). Large portions of the genome are partitioned into topological domains of chromatin interaction ranging from hundreds of kilobases to megabases (the resolution of current methods), within which the genes tend to be more coregulated (Dixon et al., 2012; Nora et al., 2012). The complex task of gene expression—ensuring the proper timing, space, and rate of expression—involves noncoding regions of the genome, chromatin modifications, and the arrangement of chromosomes and nuclear domains. Here, we review the evidence that lncRNAs are a rich source of molecular addresses in the eukaryotic nucleus.

Biogenesis and Characteristics

Efforts over the last decade revealed that a large fraction of the noncoding genome is transcribed. Extensive annotation of lncRNA has been performed in multiple model organisms (reviewed in Rinn and Chang, 2012), and there is now evidence that, whereas 2% of the genome encodes for proteins (IHGSC, 2004), primary transcripts cover 75% of the human genome, with processed transcripts covering 62.1% of the genome (Djebali et al., 2012). In this Review, we focus on a particular class of noncoding transcripts known as long noncoding RNAs (lncRNAs) and the roles that they play in nuclear organization.

lncRNAs are currently defined as transcripts of greater than 200 nucleotides without evident protein coding function (Rinn and Chang, 2012). It is important to note that lncRNA is a broad definition that encompasses different classes of RNA transcripts, including enhancer RNAs, small nucleolar RNA (snoRNA) hosts, intergenic transcripts, and transcripts overlapping other transcripts in either sense or antisense orientation. lncRNAs predominantly localize to the nucleus and have, on average, a lower level of expression than protein coding genes, although details vary for different classes (Djebali et al., 2012; Ravasi et al., 2006). Multiple studies have shown that lncRNA expression is more cell type specific than protein-coding genes (Cabili et al., 2011; Djebali et al., 2012; Ravasi et al., 2006). At the DNA and

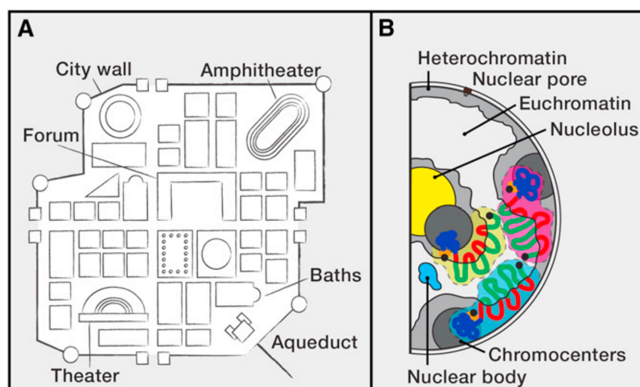

Figure 1. Comparison between a Roman City and the Cell Nucleus Reveals the Importance of Spatial Organization

(A) Depiction of the basic features of a Roman city. City walls delimit the city, with gates at the two main roads that intersect at the center of the city. The Forum was the business and political center of the city, and many buildings provided specific functions that were essential for city life.

(B) Schematic representation of the typical nuclear organization during interphase. Each chromosome occupies a discrete territory. Euchromatin localizes to the interior regions of the nucleus, and the densely compacted heterochromatin localizes near the nuclear envelope. Many specialized functions are executed in distinct regions in the nucleus, known as nuclear bodies. One example is the nucleolus, where ribosomes are assembled. Adapted from Solovei et al., 2009.

chromatin level, lncRNA loci are similar to mRNA loci, but lncRNAs show a bias for having just one intron and a trend for less-efficient cotranscriptional splicing (Derrien et al., 2012; Tilgner et al., 2012). Although lncRNAs are under lower selective pressure than protein-coding genes, sequence analysis shows that lncRNAs are under higher selective pressures than ancestral repeat sequences, which are considered to be under neutral selection. Interestingly, the promoters of lncRNAs are the region of the lncRNA gene under higher selective pressure, displaying levels of selection comparable to the promoters of protein-coding genes (Derrien et al., 2012; Guttman et al., 2009; Marques and Ponting, 2009; Ørom et al., 2010; Ponjavic et al., 2007). This analysis has also revealed a high number of correlated positions between lncRNA in sequence alignments, an observation that fits the hypothesis that lncRNAs are under selective pressure to maintain a functional RNA structure (Derrien et al., 2012). Comparison between mammalian and zebrafish lncRNAs revealed that short stretches of conserved sequence are functionally important and that location and structure of lncRNAs can be conserved, even in the absence of strong sequence conservation. The ability to induce a loss-of-function phenotype by blocking the short conserved motif in addition to the ability to rescue loss of function of two lncRNAs with the addition of human and mouse lncRNAs (Ulitsky et al., 2011) demonstrates that these "in silico" observations are of biological significance.

Sequence analysis of lncRNAs, focusing on presence and size of open reading frames as well as codon conservation frequency, has been used to exclude protein coding potential. Ribosome profiling, a method that enumerates transcripts associated with ribosomes, had detected many lncRNAs, but it was unclear whether these lncRNAs are just being scanned similarly to 5′ untranslated regions or actually are productively engaged in translation (Ingolia et al., 2011). Comparison of RNA sequencing (RNA-seq) data to tandem mass spectrometry data for two cell lines suggests that ~92% of the annotated lncRNAs do not yield detectable peptides in these cell lines (Bánfai et al., 2012; Derrien et al., 2012). Although the differences between these two studies may stem from measuring two different endpoints, they suggest that lncRNAs have low translational potential even when ribosomes attempt to decode them. Current annotations suggest that the actual number of lncRNAs exceeds that of protein coding genes (Derrien et al., 2012).

The repertoire of roles performed by lncRNAs is growing, as there is now evidence that lncRNAs participate in multiple networks regulating gene expression and function. Several characteristics of lncRNAs make them the ideal system to provide the nucleus with a system of molecular addresses. lncRNAs, unlike proteins, can function both in *cis*, at the site of transcription, or in *trans*. An RNA-based address code may be deployed more rapidly and economically than a system that relies only on proteins. lncRNAs do not need to be translated and do not require transport between the cytoplasm and the nucleus. lncRNAs can also interact with multiple proteins, enabling scaffolding functions and combinatorial control (Wang and Chang, 2011). As such, the act of transcription can rapidly create an anchor that will lead to the formation, or remodeling, of nuclear domains through the recruitment or sequestration of proteins already present in the nuclear compartment. Using lncRNAs allows cells to create addresses that are regional-, locus- or even allele-specific (Lee, 2009). At the regional level, lncRNAs can influence the formation of nuclear domains and the transcriptional status of an entire chromosome, and they can participate in the interaction of two different chromosomal regions. At a more fine-grained level, lncRNAs can control the chromatin state and activity of a chromosomal locus or specific gene. We explore each of these concepts below with recently published examples.

Locus Control of Gene Regulation

Cells can use noncoding RNAs to modulate gene expression by changing the accessibility of gene promoters. These mechanisms can be used to fine-tune gene expression in response to environmental conditions or to silence a gene as part of a developmental program.

First, the act of noncoding RNA (ncRNA) transcription itself can be purposed for regulatory function. For example, transcription through a regulatory sequence, such as a promoter, can block its function, a mechanism termed transcriptional interference (Figure 2A) first identified in yeast (Martens et al., 2004). In such instances, the lncRNA promoter is finely tuned to receive appropriate inputs to exert regulatory function; the lncRNA product is typically a faithful biomarker of transcriptional interference in action but is not required for its success. In conditions that limit vegetative growth, diploid *S. cerevisiae* cells enter sporulation, a differentiation program that results in the formation of haploid daughter cells. Entry into meiosis has catastrophic consequences in haploid cells and is therefore inhibited via a transcriptional interference mechanism. A transcription factor in haploid cells activates the expression of IRT1(SUT643),

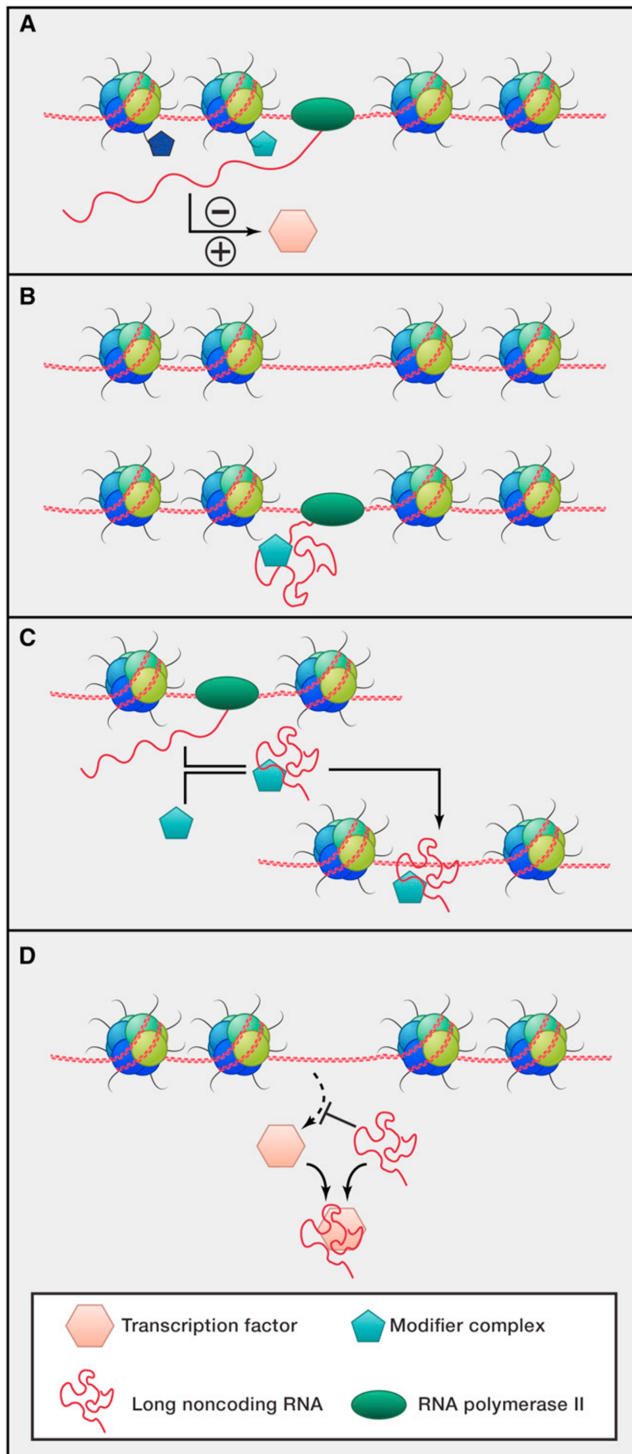

Figure 2. Functional Modules of lncRNAs in the Nucleus
(A) The act of transcription at noncoding regions can modulate gene expression through the recruitment of chromatin modifiers to the site of transcription. These complexes can create a local chromatin environment that facilitates or blocks the binding of other regulators.
(B) lncRNAs can function in *cis*, recruiting protein complexes to their site of transcription and thus creating a locus-specific address. Cells can use this mechanism to repress or activate gene expression.
(C) lncRNAs can function in *trans* and recruit protein complexes to chromatin loci away from their site of transcription.
(D) lncRNAs can bind and sequester transcription factors away from their target chromosomal regions.

a noncoding RNA that overlaps the promoter of *IME1*, the master regulator of sporulation. Transcription of IRT1 establishes a repressive chromatin state at the *IME1* promoter through the recruitment of histone methyltransferase Set2 and the histone deacetylase Set3 (van Werven et al., 2012). The use of noncoding transcription to control chromatin modification is a widespread strategy. The Set3 histone deacetylase has also been implicated in the modulation of gene induction kinetics during changes of carbon source. Transcription of ncRNAs that overlap the regulated genes leads to the establishment of H3K4me2, which recruits Set3 and leads to the deacetylation of the gene promoter. Deacetylation of the promoter results in delayed or reduced induction of the regulated genes. This mechanism is also involved in the inhibition of cryptic promoters (Kim et al., 2012). Expression of GAL10-ncRNA, driven by Reb1, leads to deacetylation across the GAL1-10 promoter, facilitating glucose repression of GAL1-10 (Houseley et al., 2008).

In mammalian imprinting, the noncoding RNA Air (also known as Airn) is expressed from the paternal chromosome and is involved in silencing the paternal alleles of multiple genes. The promoter of one of these genes, *Igf2r*, overlaps with the *Air* transcriptional unit and is silenced by transcriptional interference (Latos et al., 2012).

Transcriptional interference can also be used to activate gene expression by inhibiting the action of repressor elements, functioning as an antisilencing mechanism. In *Drosophila* embryogenesis, transcription through Polycomb response elements (PRE) alters the function of these elements, blocking the establishment of repressive chromatin (Schmitt et al., 2005).

Second, lncRNAs can silence or activate gene expression in *cis*, acting on neighboring genes of the lncRNA locus. Some of the first studied examples of lncRNA function involve dosage compensation and genomic imprinting, whereby lncRNAs provide allele-specific gene regulation to differentially control two copies of the same gene within one cell (see the Review by Lee and Bartolomei on page 1308 of this issue; Lee and Bartolomei, 2013) (Figure 2B). Several such lncRNAs are now recognized to interact with and recruit histone modification complexes, including Xist (recruits PRC2 for H3K27me3 and RYBP-PRC1 for H2A ubiquitylation) and Kcnq1ot1 (recruits G9a for H3K9me3 and PRC2) (Pandey et al., 2008; Tavares et al., 2012; Zhao et al., 2010). The Air lncRNA (the transcription of which inhibits *Igfr2*) targets G9a and H3K9me3 to silence more distantly located genes on the paternal chromosome (Nagano et al., 2008); hence, one lncRNA gene can employ multiple mechanisms to regulate nearby and distantly located genes. In genome-wide studies, numerous lncRNAs have now been found to interact with chromatin modification complexes (Guil et al., 2012; Guttman et al., 2011; Khalil et al., 2009; Zhao et al., 2010). In the plant *A. thaliana*, two cold-inducible lncRNAs, COOLAIR and COLDAIR, are embedded antisense or intronic to the flowering control locus gene *FLC*, and they help to recruit PRC2 to stably silence *FLC* in a cold-dependent manner, a key

mechanism to ensure the proper flowering time after winter termed "vernalization" (reviewed in Ietswaart et al., 2012). In an analogous fashion, DNA damage induces a lncRNA from the promoter of cyclin D1 gene (*CCND1*); this lncRNA binds to TLS protein to allosterically inhibit histone acetyltransferase in *cis*, which suppresses *CCND1* transcription (Wang et al., 2008).

DNA methylation can occur as a long-term silencing mechanism downstream of repressive histone modifications, and lncRNAs may also guide DNA methylation in addition to histone modification. The ribosomal DNA (rDNA) loci are tandemly repeated in the genome, with some copies being transcriptionally active, whereas others are silenced by DNA methylation and histone modifications. Each ribosomal DNA transcribes rRNA separated by intergenic spacers (IGSs) as a polycistronic unit, and IGSs can be processed to 150–250 nt fragments termed "promoter RNAs (pRNAs)" (reviewed in Bierhoff et al., 2010). pRNA serves as a platform to recruit the de novo cytosine methylase DNMT3 and the NoRC complex containing poly-ADP ribose polymerase-1 (PARP-1) to promote silencing of rDNA (Guetg et al., 2012; Mayer et al., 2006). Notably, a stretch of 20 nt in pRNA binds the rDNA promoter, forming a RNA:DNA:DNA triplex (Schmitz et al., 2010). This triplex structure is proposed to recruit DNMT3 and also serves as the specific recognition mechanism between lncRNA and genomic DNA—a model that likely applies to other lncRNA-DNA interactions (Martianov et al., 2007).

A distinct family of lncRNAs serves to activate gene expression. Many active enhancer elements transcribe lncRNAs, termed "eRNAs" (De Santa et al., 2010; Kim et al., 2010), and several lncRNAs are required to activate gene expression, which are termed "enhancer-like RNAs" (Ørom et al., 2010). Evf is a *cis*-acting lncRNA that is required for the activation of *Dlx5/6* genes and generation of GABAergic interneurons in vivo (Bond et al., 2009). A key mechanism of lncRNA specificity in *cis* is the higher-order chromosomal configuration (Wang et al., 2011). The noncoding RNA HOTTIP is expressed from the 5′ end tip of the *HoxA* locus and drives histone H3 lysine 4 trimethylation and gene transcription of *HoxA* distal genes through the recruitment of the WDR5/MLL complex (Wang et al., 2011). Endogenous HOTTIP is brought to its target genes by chromosomal looping, and ectopic HOTTIP only activates transcription when it is artificially tethered to the reporter gene (Wang et al., 2011). The MLL complex is also recruited to the *Hox* locus by the noncoding RNA Mistral, located between *Hoxa6* and *Hoxa7*. Mistral directly interacts with MLL1, leading to changes at the chromatin level that activate *Hoxa6* and *Hoxa7* (Bertani et al., 2011). Hence, lncRNA interaction with MLL/Trx complexes and likely additional proteins will define their function in enforcing active chromatin states and gene activation.

Third, lncRNAs can control chromatin states at distantly located genes (i.e., in *trans*) for both gene silencing and activation (Figure 2C). These lncRNAs bind to some of the same effector chromatin modification complexes but target them to genomic loci genome-wide. For instance, human HOTAIR lncRNA binds to PRC2 and LSD1 complexes and couples H3K27 methylation and H3K4 demethylation activity to hundreds of sites genome-wide (Chu et al., 2011; Tsai et al., 2010). HOTAIR is located in the *HOXC* locus and is regulated in an anatomic position-specific fashion. Linc-p21 is induced by p53 during DNA damage and recruits hnRNPK via physical interaction to mediate p53-mediated gene repression (Huarte et al., 2010). Linc-p21 also has a recently recognized role in translational control (Yoon et al., 2012). In contrast, PANDA, another lncRNA induced by p53, acts as a decoy by binding to the transcription factor NF-YA and preventing NF-YA from activating genes encoding cell death proteins (Hung et al., 2011) (Figure 2D). lncRNA-mediated activation can also occur in *trans*. Jpx, an X-linked lncRNA that activates Xist expression, is important for X chromosome inactivation in female cells, and Jpx deletion can be rescued by Jpx supplied in *trans* (Tian et al., 2010).

Nuclear Domains

The concept of lncRNA recruitment of factors to genes may be more properly considered a two-way street, with genes being moved into specific cytotopic locations by lncRNAs. One type of molecular address can be found in the formation of nuclear domains. These are regions of the nucleus where specific functions are performed. Unlike cellular organelles, these domains are not membrane delimited. They are instead characterized by the components that form them. These domains are believed to form through molecular interactions between its components. Once a stable interaction is found, the components remain associated. These domains are often formed around the sites of transcription of RNA components, which function as molecular anchors (reviewed in Dundr and Misteli, 2010). The noncoding RNA NEAT1, an essential component of the Paraspeckle, is a well-characterized example of how noncoding RNAs can function as structural components of nuclear bodies. Upon transcription of NEAT1, diffusible components of this domain nucleate at the site of NEAT1 accumulation, leading to the formation of the Paraspeckle (Figure 3A) (Chen and Carmichael, 2009; Clemson et al., 2009; Mao et al., 2011; Sasaki et al., 2009; Shevtsov and Dundr, 2011; Sunwoo et al., 2009).

Nuclear domains can be dynamically regulated in an RNA-dependent fashion. In response to serum stimulation, the demethylase KDM4C is recruited to the promoters of genes controlled by the cell-cycle-specific transcription factor E2F, where it demethylates Polycomb protein Pc2. Whereas methylated Pc2 interacts with the noncoding RNA TUG1, a component of Polycomb bodies, unmethylated Pc2 interacts with the noncoding RNA MALAT1/NEAT2, a component of interchromatin granules. Therefore, changes in the methylation status of Pc2 lead to the relocation of growth control genes from an environment that inhibits gene expression, the Polycomb body, to a domain that is permissive of gene expression, the interchromatin granule (Figure 3B). Interestingly, the reading ability of Pc2 is modulated by the noncoding RNA that it is interacting with. When bound to TUG1, Pc2 reads H4R3me^{2s} and H3K27me^{2}, whereas it reads H2AK5ac and H2AK13ac when interacting with MALAT1/NEAT2 (Yang et al., 2011). These interplays control the growth-factor-dependent expression of cell-cycle genes in vitro, but it came as a surprise that mouse knockouts of either NEAT1 or MALAT1/NEAT2 had no little overt phenotype (Eissmann et al., 2012; Nakagawa et al., 2012; Nakagawa et al., 2011; Zhang et al., 2012). Clearly, the question of redundancy or compensation in vivo needs to be addressed in the future.

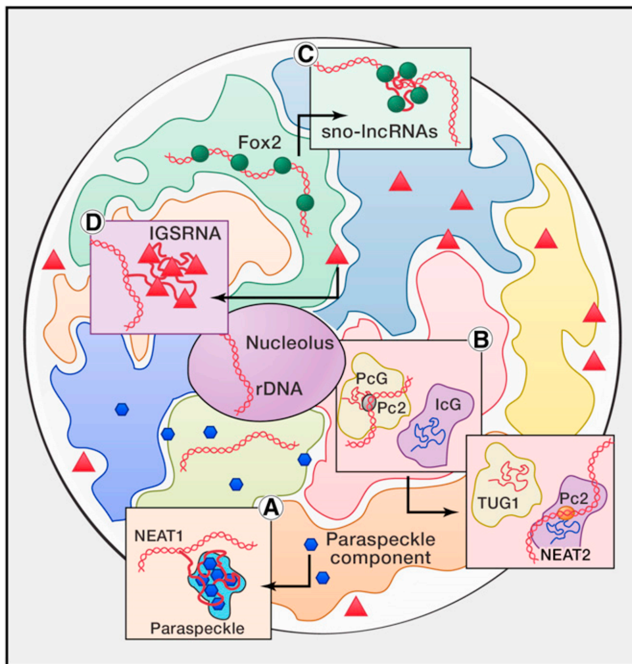

Figure 3. Schematic Representation of the Cell Nucleus, Showing the Nucleolus and Chromosomal Territories

(A) Protein components of the Paraspeckle diffused throughout the nucleoplasm aggregate upon the transcription of NEAT1, forming the Paraspeckle nuclear domain.

(B) Pc2 differentially binds MALAT1/NEAT2 or TUG1 depending on methylation status. Methylated Pc2 interacts with TUG1, bringing associated growth control genes to a repressive environment, the polycomb body (PcG). Unmethylated Pc2 interacts with MALAT1/NEAT2 at the interchromatin granule (ICG), where gene expression is permitted.

(C) Expression of lncRNAs with snoRNA ends from the Prader-Willi syndrome locus functions as a sink for the FOX2 protein, leading to redistribution of this splicing factor in this nuclear region.

(D) In response to cellular stress, transcription of specific IGSRNAs leads to the retention of targeted proteins at the nucleolus. Different types of stress lead to the retention of different proteins through the expression of specific noncoding RNAs.

Unusual processing mechanisms may explain the localization activity of certain lncRNAs. An imprinted region in chromosome 15 (15q11-q13) that had been implicated in Prader-Willi syndrome (PWS) hosts multiple intron-derived lncRNAs with small nucleolar RNAs at their ends—so called "sno-lncRNAs." It is probable that the presence of structured snoRNAs at the ends of lncRNAs stabilizes these molecules, which have no 5′ cap or polyA tail. These RNAs are retained in the nucleus and localize to, or remain near, their sites of transcription. Knockdown of sno-lncRNAs has little effect on the expression of nearby genes, suggesting that it does not affect gene expression in *cis*. Instead, these sno-lncRNAs seem to create a "domain" where the splicing factor Fox2 is enriched. These sno-lncRNAs contain multiple binding sites for Fox2, and altering the level of sno-lncRNAs led to a redistribution of Fox2 in the nucleus and changes in mRNA splicing patterns. Hence, the sno-lncRNAs appear to function as Fox2 sinks, participating in the regulation of splicing in specific subnuclear domains (Yin et al., 2012) (Figure 3C). Similarly, formation of a blunt-ended triplex RNA structure at the 3′ end of MALAT1/NEAT2 lncRNA, which lacks a polyA tail, stabilizes the lncRNA and presumably limits its export to the cytoplasm (Brown et al., 2012; Wilusz et al., 2012). Viral nuclear lncRNAs have also adapted this strategy and hide their 3′ polyA tails in a triplex RNA structure to prevent decay (Mitton-Fry et al., 2010; Tycowski et al., 2012).

Gene Control through Sequestration

In contrast to the model of nuclear domains that concentrate and thereby facilitate molecular interactions, spatial control can also separate reactants until the moment is right. For example, certain environmental stresses trigger the retention of select proteins in the nucleolus away from their normal site of action. The retention at the nucleolus requires a signal sequence and the expression of specific noncoding RNAs expressed from the large intergenic spacer (IGS) of the rDNA repeats. IGS ncRNAs turn out to gate the responses to cellular stress. Unique IGS ncRNAs are transcriptionally induced by specific stressors, functioning as baits for proteins with specific signal sequences. Interfering with a specific IGSRNA does not affect the function of other IGSRNAs (Audas et al., 2012) (Figure 3D).

In *S. pombe*, both mRNAs and lncRNAs function together to form heterochromatin and sequester genes in the control of meiosis. During vegetative growth, the expression of meiotic genes is repressed through selective elimination of meiotic mRNAs. Meiotic genes contain within their transcripts a region known as determinant of selective removal (DSR) that determines their degradation. This sequence is recognized by Mmi1, which promotes both mRNA degradation (Harigaya et al., 2006) as well as formation of facultative heterochromatic islands (Zofall et al., 2012). Hence, aberrant nascent mRNAs can function in an lncRNA-like fashion to tether the formation for heterochromatin. Furthermore, during vegetative growth, Mei2p, an RNA-binding protein that is crucial for entry in meiosis, is kept in an inactive form. When cells commit to the meiosis expression program, Mei2p accumulates in its active form and sequesters Mmi1 to a structure known as Mei2 dot, where Mmi1 function is inhibited. The Mei2 dot forms at the *sme2* locus at the site of transcription of two noncoding RNAs, meiRNA-S and meiRNA-L, which are necessary for the formation of the Mei2 dot structure and, therefore, entry in meiosis (Yamamoto, 2010).

Higher-Order Chromosomal Interactions

An intriguing possibility is that lncRNAs can regulate the three-dimensional structure of the chromosomes by facilitating the interaction of specific chromosomal loci. The act of transcription itself can influence gene expression and genome organization by promoting chromatin modifications, by recruiting gene active regions to common transcription factories, or by exposing the DNA strands to enzymatic activity. Hence, the presence of multiple lncRNA genes in a region may help chromosomal loci adopt distinct conformation with transcriptional activation. For example, in the *Hox* loci, collinear expression of *Hox* mRNA genes and *Hox* lncRNAs along the chromosome is associated with the progressive recruitment of those chromosomal segments into a tightly interacting domain that is distinct from the transcriptionally silent portion of the loci (Noordermeer

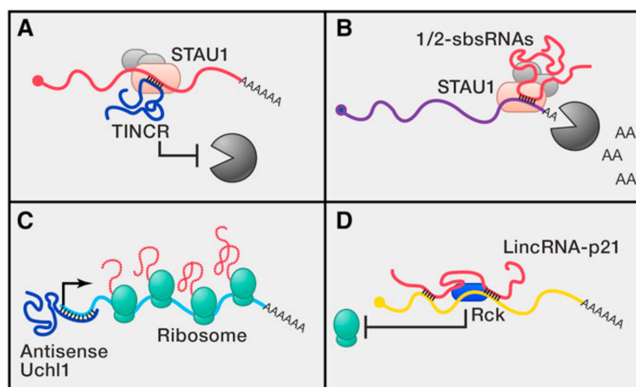

Figure 4. lncRNAs Regulate Gene Expression in the Cytoplasm
(A) The lncRNA TINCR interacts with STAU1 and target mRNAs containing the TINCR box motif, promoting their stability.
(B) lncRNAs of the 1/2-sbsRNAs class hybridize with 3′-UTR-containing Alu elements and promote the degradation of these target mRNAs.
(C) Under stress conditions, the lncRNA antisense to *Uchl1* moves from the nucleus to the cytoplasm and binds the 5′ end of the *Uchl1* mRNA to promote its translation under stress conditions.
(D) lincRNA-p21 interacts with and targets RcK to mRNAs, resulting in translation inhibition.

et al., 2011; Wang et al., 2011). A similar phenomenon was first appreciated in the β-globin locus, and intergenic transcripts from its locus control regions (Ashe et al., 1997). Transcription-coupled looping is likely to be related to the fact that the Mediator complex that links transcription factors to basal transcription machinery promotes long-range enhancer-promoter interactions (Kagey et al., 2010). A similar transcription-directed mechanism has also been proposed to guide DNA recombination of lymphocyte receptor genes over megabases (Verma-Gaur et al., 2012). The lncRNA transcripts are useful readouts of the chromosomal configuration but are not necessarily required for the chromosomal interactions.

lncRNAs can also regulate chromosome structure through direct mechanisms. High-throughput chromosomal conformation assays revealed that the active and inactive X chromosomes adopt quite distinct conformations. The inactive X (Xi) is coated by the Xist lncRNA, which is required for choosing the inactive X chromosome. Importantly, conditional knockout of Xist has demonstrated that the folding of inactive X requires the Xist RNA. After Xist deletion, the Xi chromosome adopts a conformation that is more similar to that of the active X chromosome (Xa) without reactivation of Xi gene expression. Hence, Xist appears to regulate X chromosome structure through mechanisms other than the relocation of active genes to transcriptional factories (Splinter et al., 2011). One intriguing clue is that conditional Xist deletion also led to loss of PRC2 and H3K27me3 marks. The conformations of the two X chromosomes appear to be regulated by distinct mechanisms because PRC2 is dispensable for the topological domains of Xa (Nora et al., 2012). Whether one or several Xa-expressed lncRNA controls Xa conformation remains to be seen.

lncRNAs can also regulate the interaction between chromosomes, a concept that is exemplified by *S. pombe* meiosis. In order for chromosomes to properly segregate in meiosis and prevent aneuploidy, homologous chromosomes must interact and generate stable associations. The *sme2* locus plays a key role in the mutual identification of homologous chromosomes during meiosis, in addition to its role in the mitosis/meiosis switch discussed above. The meiRNA-L transcript accumulates at the *sme2* locus and is necessary for the robust chromosomal pairing (Ding et al., 2012). These studies suggest that noncoding RNAs can be components of a *cis*-acting pairing factor that allows homologous chromosomes to identify each other.

Cytoplasmic Functions

The ultimate function of mRNAs is to be translated, and like other steps of gene expression, multiple layers of posttranscriptional regulation exist in the cytoplasm (Figure 4). lncRNAs can also "identify" mRNAs in the cytoplasm and modulate their life cycle. Recent works demonstrated that lncRNAs impact both the mRNA half-life and translation of mRNAs. The lncRNA TINCR (terminal differentiation-induced ncRNA) is induced during epidermal differentiation and is required for normal induction of key mediators of epidermal differentiation. TINCR localizes to the cytoplasm, where it interacts with Staufen 1 protein (STAU1) to promote the stability of mRNAs containing the TINCR box motif (Kretz et al., 2013) (Figure 4A). Hence, the TINCR mechanism is the diametric opposite of posttranscriptional silencing by small regulatory RNAs like siRNA or miRNAs. STAU1 can also be programmed by other lncRNAs to facilitate mRNA degradation. The half-STAU1-binding site RNAs (1/2-sbsRNAs) contain Alu elements that bind to Alu elements in the 3′UTR of actively transcribed target genes, generating a STAU1-binding site. These mRNAs are therefore identified as STAU1-mediated messenger RNA decay (SMD) targets (Gong and Maquat, 2011) (Figure 4B). In addition, a recently identified class of lncRNA impacts gene expression by promoting translation of targets mRNAs. Expression of antisense *Uchl1* RNA leads to an increase in Uchl1 protein level without any change at the mRNA level. Antisense *Uchl1* lncRNA is composed by a region that overlaps with the first 73 nucleotides of *Uchl1* and two embedded repetitive sequences, one of which (SINEB2) is required for the ability of the lncRNA to induce protein translation. Under stress conditions in which cap-dependent translation is inhibited, antisense *Uchl1* lncRNA, previously enriched in the nucleus, moves into the cytoplasm and hybridizes with *Uchl1* mRNA to enable cap-independent translation of *Uchl1*. In other words, the lncRNA acts like a mobile internal ribosomal entry element to promote selective translation. Other SINEB2-containing antisense lncRNAs may function in a similar way (Carrieri et al., 2012) (Figure 4C). Conversely, lincRNA-p21 can inhibit the translation of target mRNAs. In the absence of HuR, lincRNA-p21 is stable and interacts with the mRNAs CTNNB1 and JUNB and translational repressor Rck, repressing the translation of the targeted mRNAs (Yoon et al., 2012) (Figure 4D). These emerging examples illustrate that lncRNAs can provide a rich palette of regulatory capacities in the cytoplasm.

Human Diseases

Considering the wide range of roles that lncRNAs play in cellular networks, it is not surprising that noncoding RNAs have been implicated in disease. Genome-wide association studies have

revealed that only 7% of disease or trait-associated single-nucleotide polymorphisms (SNPs) reside in protein-coding exons, whereas 43% of trait-/disease-associated SNP are found outside of protein-coding genes (Hindorff et al., 2009). In addition to the example of sno-lncRNAs in Prader-Willi syndrome discussed above, several recent discoveries of lncRNAs in Mendelian disorders illustrate the emerging recognition of lncRNAs in human diseases.

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common myopathy and is predominantly caused by a contraction in copy number of the D4Z4 repeats mapping to 4q35. The D4Z4 repeat is the target of several chromatin modifications, including H3K9me3 and H3K27me3, which are reduced in FSHD patients. Cabianca et al. found that a long array of D4Z4 repeats recruit Polycomb complexes to promote the formation of a repressive chromatin state that inhibits the expression of genes at 4q35. Loss of D4Z4 repeats results in derepression of DBE-T, a novel lncRNA that functions in *cis* and localizes to the FSHD locus. DBE-T recruits ASH1L (a component of MLL/TrX complex), leading to improper establishment of active chromatin and expression of genes from 4q35 (Cabianca et al., 2012). Hence, DBE-T is a lncRNA that functions as a locus control element by promoting active chromatin domain, and FSHD results from lncRNA "promoter mutations" that perturb DBE-T regulation.

HELLP syndrome (*h*emolysis, *e*levated *l*iver enzymes, *l*ow *p*latelets) is a recessively inherited life-threatening pregnancy complication. Linkage analysis narrowed the HELLP locus to a gene desert between *C12orf48* and *IGF1* on 12q23.2, where a single 205 kb capped and polyadenylated lncRNA is transcribed (van Dijk et al., 2012). Knockdown of this lncRNA revealed a role in the transition from G2 to mitosis and trophoblast cell invasion, although the precise mechanism is still unclear. Notably, morpholino oligonucleotides complementary to the mutation site in HELLP lncRNA boosted lncRNA level and reversed the gene expression and cell invasion defects.

Similarly, deletions in a coding-gene desert at 16q24.1 lead to *a*lveolar *c*apillary *d*ysplasia with *m*isalignment of *p*ulmonary *v*eins (ACD/MPV) (Szafranski et al., 2013). This region contains a distant enhancer of *FOXF1*, a key regulator of lung development. This enhancer element interacts with *FOXF1* in human pulmonary microvascular endothelial cells, but not in lymphoblasts, suggesting that FOXF1 expression in the lung endothelium is regulated at the chromatin structure levels. In addition to transcription-factor-binding sites, the focal deletion includes two lncRNA expressed specifically in the lung. An intriguing possibility is that the expression of these lncRNAs, which happens specifically in the lung, contributes to the establishment of a chromatin loop that brings the enhancer in close proximity to *FOXF1*.

Chromosomal translocations lead to inheritable structural and genetic changes and, as such, are relevant causes of genetic disease. One way that chromosomal translocations can lead to disease is through disruption of the higher-order chromatin organization and the *cis*-regulatory landscape. Recently, two different translocations have been identified in brachydactyly type E (BDE) that implicate lncRNA dysregulation (Maass et al., 2012). These translocations affect a regulatory region that interacts in *cis* with *PTHLH* and in *trans* with *SOX9*. Interestingly, this region is home to a lncRNA whose expression is important for the proper expression of *PTHLH* and *SOX9*. Depletion of this lncRNA (*DA125942*) resulted in downregulation of *PTHLH* and *SOX9*. The lncRNA interacts with both loci, and the occupancy is reduced in chromatin originated from BDE patients. This study demonstrates how lncRNAs and chromatin higher-order organization collaborate in the regulation of gene expression.

Recognition of the roles of lncRNAs in human disease has unveiled new diagnostic and therapeutic opportunities. lncRNAs are expressed in a more tissue-specific fashion than mRNA genes, a pattern that has been found to hold true in pathologic states such as cancer (Brunner et al., 2012). lncRNA measurements could hence trace cancer metastases or circulating cancer cells to their origins. In addition, a strong connection between lncRNAs and cancer has been clearly established, as many lncRNAs are dysregulated in human cancers. The lncRNA HOTAIR in overexpressed in breast, colon, pancreas, and liver cancers, and overexpression of HOTAIR has been shown to drive breast cancer metastasis in vivo (Gupta et al., 2010; Gutschner and Diederichs, 2012). lncRNAs appear to be more structured and stable than mRNA transcripts, which facilitate their detection as free nucleic acids in body fluid such as urine and blood—knowledge already put to good use in clinically approved tests for prostate cancer (Fradet et al., 2004; Shappell, 2008; Tinzl et al., 2004). Aberrant lncRNAs can be knocked down in vivo using oligonucleotide "drugs" (Modarresi et al., 2012; Wheeler et al., 2012), which should spur advance in lncRNA genetics and therapeutics.

Conclusions

lncRNAs are well poised to be molecular address codes, particularly in the nucleus. On the one hand, transcription of lncRNAs is often exquisitely regulated, reflecting the particular developmental stage and external environment that the cell has experienced. On the other, the capacity of lncRNAs to function as guides, scaffolds, and decoys endows them with enormous regulatory potential in gene expression and for spatial control within the cell. These outstanding properties of long RNAs have already been leveraged to make designer RNA scaffolds for synthetic cell circuits (Delebecque et al., 2011). Many questions remain to be addressed in this rapidly expanding field. First, the in vivo function of most lncRNAs has not been determined. An extensive catalog of lncRNAs has recently been described available for several model organisms (Nam and Bartel, 2012; Pauli et al., 2012; Ulitsky et al., 2011), opening the door of a wide array of powerful techniques to be used in the in vivo study of lncRNAs that will complement the study of human lncRNAs. In addition, detailed knowledge of structure-function relationship in lncRNAs is still lacking, which prohibits the de novo prediction of lncRNA domains and functions that we take for granted in protein-coding transcripts. New technologies to deconvolute RNA structure and function (Martin et al., 2012; Wan et al., 2012), probe RNA-chromatin interactions (Chu et al., 2011; Simon et al., 2011), and track RNA movement in real time (Paige et al., 2011) will be crucial for understanding lncRNAs and realizing their therapeutic potential.

ACKNOWLEDGMENTS

We thank members of the Chang lab for discussion and apologize to colleagues whose works are not discussed due to space limitation. We acknowledge support from NIH and California Institute for Regenerative Medicine (H.Y.C.). P.J.B. is the Kenneth G. and Elaine A. Langone Fellow of the Damon Runyon Cancer Research Foundation. H.Y.C. is an Early Career Scientist of the Howard Hughes Medical Institute. H.Y.C. is on the Scientific Advisory Board of RaNA Therapeutics, which works on long noncoding RNAs.

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