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Function of lncRNAs and approaches to lncRNA-protein interactions

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Long non-coding RNAs (lncRNAs), which represent a new frontier in molecular biology, play important roles in regulating gene expression at epigenetic, transcriptional and post-transcriptional levels. More and more lncRNAs have been found to play important roles in normal cell physiological activities, and participate in the development of varieties of tumors and other diseases. Previously, we have only been able to determine the function of lncRNAs through multiple mechanisms, including genetic imprinting, chromatin remodeling, splicing regulation, mRNA decay, and translational regulation. Application of technological advances to research into the function of lncRNAs is extremely important. The major tools for exploring lncRNAs include microarrays, RNA sequencing (RNA-seq), Northern blotting, real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR), fluorescence *in situ* hybridization (FISH), RNA interference (RNAi), RNA-binding protein immunoprecipitation (RIP), chromatin isolation by RNA purification (ChIRP), crosslinking-immunopurification (CLIP), and bioinformatic prediction. In this review, we highlight the functions of lncRNAs, and advanced methods to research lncRNA-protein interactions.

lncRNA, function, RNA-protein interaction

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Long non-coding RNAs (lncRNAs), which are widely distributed in mammals, are a class of RNA molecules with more than 200 bases that function as RNAs with little or no protein-coding capacity [1]. Large-scale analyses of the mammalian transcriptome have shown that the number and types of lncRNAs far exceed those of protein-coding mRNAs [2–5]. However, a small proportion of lncRNAs have been reported to have biological functions. Increasing evidence indicates that lncRNAs play important roles in a variety of biological processes through complicated mechanisms [6–10]. Aberrant expression of lncRNAs has been shown to be associated with several types of cancer, Alzheimer's disease, Huntington's disease and cardiovascular diseases [11–22]. Therefore, understanding the biological roles of lncRNAs will advance our understanding of this frontier of molecular biology.

Despite their wide distribution within mammals, the functions of lncRNAs remain poorly understood. Establishment of research technologies to decode the functions of lncRNAs is an area of focus in genomics research. Here, we will highlight methods to research interactions between lncRNAs and protein.

1 Characteristics of lncRNAs

As with the vast majority of gene transcripts, lncRNAs are mRNA-like transcripts ranging from 200 nucleotides (nt)

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to >100 kilobases (kb), with little or no protein-coding capacity [1]. These lncRNAs may be located within the cellular nucleus or cytoplasm, may or may not be polyadenylated, and are transcribed by RNA polymerase from either strand within a coding locus [3,5]. The biogenesis process of many currently identified lncRNAs is similar to mRNAs [23]. To facilitate research and academic exchange, many relevant databases providing expression and other data concerning lncRNAs have been built. The data collected in these databases come from GenBank and published papers [24–37] (Table 1).

Unlike protein-coding genes, which arise by a process of wholesale or partial duplication and subsequent sequence divergence, most lncRNAs exhibit a low degree of evolutionary constraint and therefore evolve very differently [38]. There are various sources for the emergence of lncRNAs [38]: (i) frame disruption of protein-coding genes can form lncRNAs that incorporate some previous coding sequences. For example, it has been identified that the exons and promoter of the Xist gene derive from the protein-coding gene *Lnx3* [39]; (ii) chromosomal rearrangements could cause lncRNAs to emerge from previously untranscribed sequences; (iii) retro-transposition of ncRNA in the duplication process can generate a reverse transcription gene or a retropseudogene; (iv) local tandem repeat sequences may also generate a new lncRNA. This phenomenon is observed in the 5′ regions of Kcnq1ot1 and Xist transcripts; (v) insertion of a transposable element can also generate lncRNAs, such as the transcripts of brain cytoplasmic RNA 1 (BC1) and brain cytoplasmic RNA 200-nucleotide (BC200). According to their positional relationship with protein-coding genes, lncRNAs can be divided into the following categories [40]: (i) sense; (ii) antisense: the lncRNA transcript overlaps the exons of another transcript; (iii) bidirectional: expression of lncRNAs is in the same direction as a neighboring coding transcript in the same chain; (iv) intronic: lncRNAs derive wholly from introns of another transcript; (v) intergenic: lncRNAs lie within the interval between two genes.

lncRNAs are widely distributed in different tissues, and some lncRNAs are found to be preferentially expressed in specific tissues [41]. The cellular localization of lncRNAs is also varied. lncRNAs can be observed in a full range of sub-cellular compartments, such as in the nucleus, cytoplasm, or at one or more foci of cells [42]. However, localization patterns of some lncRNAs are unusual or unique. For example, Gomafu is exclusively located in nuclear speckles [43]. The location of a lncRNA may imply its function.

Previous studies on ribozymes have shown that particular structures within these RNAs are important for their functions [44]. Similarly, distinct RNA structures are critical for the function of the steroid receptor RNA [45]. Therefore, prediction of stem-loop secondary structures is helpful for identifying functions of lncRNAs. Approaches that predict the structure of lncRNAs have yet to be developed [46]. A widely used computer program, Mfold, was established to predict RNA secondary structure [47,48]. Some newer programs, such as PPfold and CompaRNA, have been established [49,50]. The complete repertoire of structured ncRNA elements remains to be determined.

In summary, we conclude that the characteristics of lncRNAs consist of multiple types, modes of action and numbers. Although the characteristics of lncRNAs are complicated, it has been shown that they play similar roles in regulating gene expression [51]. Recent studies have explored several functions and mechanistic roles of these lncRNAs. An emerging model is that that lncRNAs might regulate specificity through assembling distinct regulatory components, including diverse combinations of proteins, RNA, and DNA interactions [52].

2 Biological functions of lncRNAs

lncRNAs, initially considered to be non-functional byproducts of RNA polymerase II transcripts, have been argued to be spurious transcriptional noise [53]. However, accumulated evidence suggests that lncRNAs have a great diversity of important functions in cellular development and metabolism, including genetic imprinting, genome rearrangement,

Table 1 Publicly available lncRNA online databases

Database	Species	Contain miRNA and snoRNA a)	Website	References
lncRNAdb	Multiple	N	http://www.lncrnadb.org/	[27]
fRNA	Multiple	Y	http://www.ncrna.org/	[26]
Noncode	Multiple	Y	http://www.noncode.org/	[29]
Rnadb	Multiple	Y	http://research.imb.uq.edu.au/rnadb/	[30,31]
Non-coding	Multiple	Y	http://www.man.poznan.pl/5SData/ncRNA/index.html	[24]
NRED	Human Mouse	N	http://jsm-research.imb.uq.edu.au/nred/cgi-bin/ncrnadb.pl	[25]
Rfam	Multiple	Y	http://rfam.sanger.ac.uk/	[32–34]
ncFANs	Human, mouse	N	http://www.noncode.org/ncFANs/	[28]
lncRNADisease	Human	N	http://cmbi.bjmu.edu.cn/lncrnadisease	[35]
LNCipedia	Human	N	http://www.lncipedia.org	[37]
ChIPBase	Multiple	Y	http://deepbase.sysu.edu.cn/chipbase/	[36]

a) Y, this database contains miRNA and snoRNA besides lncRNA. N, this database does not contain miRNA and snoRNA.

chromatin modification, regulation of the cell cycle, transcription, splicing, mRNA decay, and translation [8,9,54–67] (Table 2). Thousands of lncRNAs have been found to be involved in mammalian gene regulation [10], using similar mechanisms. A wealth of information about lncRNAs has been discovered as time progresses, especially in recent years [8,10,15,63,68–84]. Various mechanisms of action of lncRNAs have been discovered, increasing our understanding of lncRNAs (Figure 1). Generally, lncRNAs regulate gene expression at three levels: epigenetics, transcriptional regulation and post-transcriptional regulation [40].

2.1 Imprinted lncRNA genes

Intriguingly, a high proportion of ncRNA genes are located in imprinted regions. Over the past 10 years, a number of lncRNAs, such as H19, Xist and Meg3, were found to be located in imprinted genomic loci [18,85,86]. Aberrant gene expression resulting from loss of imprinting is a key feature of cancer [87,88].

H19 is a 2.3 kb lncRNA that is exclusively maternally expressed [89]. *H19* and its reciprocally imprinted neighbor insulin-like growth factor 2 (*IGF2*) are both located at 11p15.5, and the distance between these two genes is only about 90 kb [68,90]. *H19* is highly expressed during vertebrate embryo development, but is rapidly down-regulated in most tissues shortly after birth [90]. DNA methylation is involved in the establishment and maintenance of genomic imprinting, which occurs in the imprinting control region (ICR). The ICR exists between *H19* and *IGF2*. As the ICR on the maternal allele is not methylated, it can be bound by the transcription factor CTCF (CCCTC-binding factor), resulting in expression of *H19* and silencing of *IGF2*. The opposite expression of these two genes is seen on the paternal allele, because the ICR is methylated [56,91,92].

In female cells, the 17 kb lncRNA XIST plays an important role in X-chromosome inactivation [69]. The X-inactivation center (Xic) controls the silencing of one of the two X chromosomes in female cells, thereby maintaining dosage compensation [57]. A 1.6 kb lncRNA called Repeat A (Rep A) is encoded by the 5′ end of the *XIST* gene. Rep A can directly interact with the Polycomb repressive complex 2 (PRC2), move to *Xic*, and activate *XIST* expression [93,94]. Transcription of *XIST* physically coating one X chromosome, accompanied by a large number of methylated histones, may eventually lead to X-chromosome inactivation [57]. Additionally, Xist is regulated by Tsix, Xist's antisense counterpart that antagonizes Xist [58,70].

Table 2 Partial biological function of lncRNAs

lncRNA	Size (kb)	Biological function
H19	2.3	Genomic imprinting
RepA	1.6	X chromosome inactivation
HOTAIR	2.2	Recruitment and binding of chromatin remodeling complexes to HOXD
lincRNA-p21	3.1	Represses many genes transcriptionally regulated by P53
Gas5	0.6	Bait of glucocorticoid receptor
PANDA	1.5	Limits apoptosis through binding to transcription factor NF-YA
1/2-sbsRNA	0.532	Mediates decay of mRNA
BACE1-AS	2.0	Increases stability of mRNA
MALTA1	6.9	Controls cell cycle progression by regulating B-MYB
LALR1	1.1	Accelerates hepatocyte proliferation by activating Wnt/β-Catenin signaling
TINCR	3.7	Controls human epidermal differentiation by interacting with a range of differentiation mRNAs
slincRAD	136	Causes lipid accumulation in abiogenesis

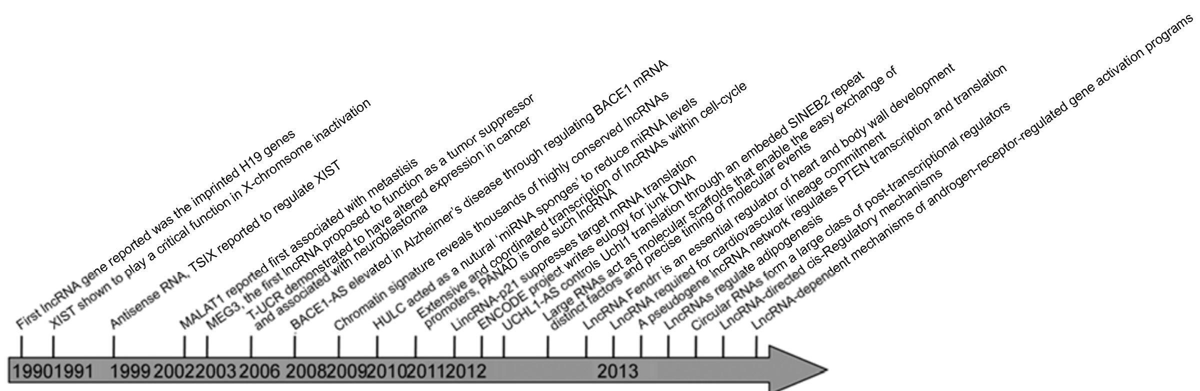

Figure 1 Timeline of important lncRNA discoveries (modified from reference [85]).

2.2 Chromatin modification by lncRNAs

By formatting silencing complexes that can result in chromatin remodeling, lncRNAs can precisely control transcription [95]. Recruitment of chromatin remodeling complexes is the main mechanism of gene regulation mediated by lncRNAs. A variety of molecular investigations authenticate the association between lncRNAs, such as HOTAIR, Kcnq1ot1, and Air, and chromatin remodeling complexes, such as PRC1 and PRC2, which mediate ubiquitination and histone methylation, respectively [54,96–101].

At the *Kcnq1* locus, the lncRNA Kcnq1ot1 interacts with members of the PRC1 and PRC2 complexes [96,99,102]. At the *Igf2r* locus, Air associates with the histone methyltransferase G9a [99]. At the *HOXD* locus, HOTAIR recruits PRC2 to induce silencing of target genes [97]. Further studies imply that association with lncRNAs is essential for target specificity of these chromatin remodeling complexes [93,97,103,104].

HOTAIR is a 2.2 kb lncRNA located in the *HOXC* locus on chromosome 12q13.13 [97]. This lncRNA has been demonstrated to interact with PRC2 and LSD1/CoREST/REST complexes [54]. As a molecular scaffold, the 5′ region of HOTAIR binds to PRC2, which is responsible for histone methylation, and its 3′ region binds to LSD1, a histone demethylase [54,105]. HOTAIR functions in the recruitment and binding of chromatin remodeling complexes to the HOXD locus on chromosome 2, resulting in re-targeting of PRC2. The consequence is the transcriptional silencing of a roughly 40 kb region of HOXD [97]. Enforced expression of HOTAIR has been shown to induce re-targeting of PRC2, leading to altered gene expression and chromatin state, and to promote cancer metastasis [14].

2.3 lncRNAs regulate cell cycle and apoptosis

It has been shown that lncRNAs also have a role in cell growth control, mainly through regulation of the cell cycle and apoptosis. Growth arrest-specific 5 (Gas5) non-coding RNA accumulates in growth-arrested cells [106] and sensitizes mammalian cells to apoptosis by suppressing genes responsive to glucocorticoid [16,60]. Gas5 interacts with the DNA-binding domain (DBD) of the glucocorticoid receptor (GR) through a decoy "glucocorticoid response element" (GRE), and suppresses GR-induced transcriptional activity by competing with the GREs of target genes.

Long intergenic non-coding RNA-p21 (lincRNA-p21), activated by p53, plays an important role in the p53 pathway, triggering apoptosis. Transcriptional repression by lincRNA-p21 is mediated through binding to heterogenous nuclear ribonucleoprotein-K (hnRNP-K). This interaction is required for directing hnRNP-K to repressed genes [9].

PANDA lncRNA, located approximately 5 kb upstream of the *CDKN1A* transcriptional start site (TSS), is transcribed antisense to CDKN1A and is involved in the DNA-damage response. After DNA damage, p53 activates transcription of *CDKN1A* as well as PANDA and lincRNA-p21. Binding of PANDA to the transcriptional factor NF-YA blocks apoptosis. These three activated genes function synergistically to arrest cell cycle and survival [8].

2.4 lncRNAs regulate mRNA decay

The abundance of mRNA has a direct relationship to the protein output, with the main factors affecting mRNA abundance being transcriptional intensity and degradation rate. The amount of mRNA transcription is mainly determined by transcriptional regulation and post-transcriptional processes, and mRNA is decayed through a variety of pathways. Staufen 1 (STAU1)-mediated mRNA decay (SMD) involves decay of active mRNAs. Base-pairing between Alu elements in the Half-STAU1-binding site RNA (1/2-sbsRNA), and Alu elements in the 3′UTR of the SMD target, can form STAU1-binding sites. This results in the activation of STAU1, and binding of STAU to mRNA. This finding uncovers a novel strategy that is used to recruit proteins to mRNA and mediate mRNA decay [55].

2.5 lncRNAs regulate protein translation

Recent studies reveal that lncRNAs are also involved in protein translation. A lncRNA antisense to mouse ubiquitin carboxy-terminal hydrolase L1 (UCHL1) can specifically increase UCHL1 protein synthesis. Its activity depends on an embedded SINEB2 repeat [74]. BACE-AS is a natural antisense transcript of BACE1 (β-site APP cleaving enzyme 1). Base-pairing between BACE-AS and BACE1 mRNA can enhance the stability of BACE1 mRNA, thereby increasing the amount of the BACE1 protein, which is involved in Alzheimer's disease [63].

3 Research strategies of lncRNAs

A variety of research methods to explore the function and molecular mechanisms of action of lncRNAs have gradually become areas of much interest. Currently, essential schemes for exploring the function of lncRNAs include (i) high-throughput analysis of lncRNA expression; (ii) verification of high-throughput data; (iii) research into the lncRNAs-protein interactions.

3.1 High-throughput analysis of lncRNA expression

Microarrays and RNA-seq are effective tools for high-throughput analysis of lncRNA expression [107,108]. Ørom and colleagues [7] have detected 3019 kinds of lncRNAs through microarrays for profiling lncRNAs in a variety of human cell lines. Ng et al. [109] have adopted a custom-designed microarray to identify expression profiles of

human embryonic stem cells (hESCs). They identified some hESC-specific lncRNAs involved in the maintenance of pluripotency. Moreover, microarrays have been used to identify the lncRNA expression profiles in breast cancer [110], pancreatic cancer [111], hepatocellular carcinoma (HCC) [112], and peripheral blood mononuclear cells [113]. RNA-seq is used to perform transcriptome analysis. The advantage of this method is low background signal and possible discovery of new transcripts [108,114,115]. With the aid of RNA-seq, Lin et al. [116] have found that some lncRNAs are involved in neuropsychiatric disorders. Huang et al. [117] have conducted RNA-seq to analyze transcriptome complexity in HCC. Because of the difficulty of identifying lncRNAs from high-throughput RNA-sequencing data, Sun et al. [118] have implemented iSeeRNA, which is a support vector machine-based classifier for identification of lincRNA.

3.2 Verification of high-throughput data

Northern blots, qRT-PCR, FISH, and RNAi are used to verify the authenticity of high-throughput data. Furuno et al. [119] have conducted Northern blots and qRT-PCR to validate the existence of eight novel lncRNAs. Similar to Northern blotting, the underlying principle of FISH is molecular hybridization [120]. Researchers have used this technology to detect and locate lncRNAs such as Xist, MALAT1, MEN ε/β, and Kcnq1ot1 [62,121–123]. For example, Chureau et al. [121] have analyzed the location of Xist in female ES cells. RNAi silencing of specific lncRNAs has been widely used in cell culture and living organisms [124]. HOTAIR is a molecular scaffold that has binding domains for PRC2 and LSD1 in the 5′ region and 3′ region, respectively. Tsai et al. [54] have found that RNAi of HOTAIR can abrogate this interaction, suggesting that HOTAIR is essential for bringing about this interaction. Depletion of gadd7 by shRNA can significantly delay and diminish endoplasmic reticulum stress induced by reactive oxygen species [125]. Chakraborty et al. [126] have reported combined RNAi and localization analysis of lncRNAs (c-KLAN) for functionally dissecting lncRNAs. Using this technique, they have identified transcripts involved in regulating the identity of mouse ES cells. The rate and stability of the base-pairing reaction is enforced by using locked nucleic acids (LNAs) formed of standard base pairs [127]. Sarma et al. [128] have also used LNAs to reveal the relative location of Xist.

3.3 Research on lncRNA-protein interactions

It is currently believed that lncRNAs conduct their regulatory functions in the form of RNA-protein complexes, such as chromatin-modifying complexes, transcriptional factors and RNP complexes. Therefore, techniques to study lncRNA-protein interactions can uncover the mechanisms of action of lncRNAs in biological processes. Proteins interact with RNA through manners similar to protein-DNA interactions. The most common methods and the latest reported methods for studying protein-RNA interactions are discussed below. We have summarized the characteristics of methods for identifying lncRNA-protein interactions in Table 3.

3.3.1 RNA-pulldown assay

An RNA-pulldown assay, which used high affinity tags such as biotin, selectively extracts a protein-RNA complex from a sample *in vitro*. RNA probes can be biotinylated, incubated with cell lysate or recombinant protein, and then purified with streptavidin agarose or magnetic beads. The proteins are detected by Western blotting or mass spectrometry.

This is a preliminary *in vitro* method of stimulating interaction between protein and RNA. Rinn et al. [9] have used this method to identify proteins that are possibly associated with lincRNA-p21.

3.3.2 RIP and RIP-chip/seq

RNA immunoprecipitation (RIP) is a powerful technique that can be used to detect the interaction between individual proteins and specific ncRNA molecules *in vivo* [129]. Following immunoprecipitation of the protein of interest, relevant RNAs can be isolated and quantitated by qRT-PCR.

Table 3 Characteristics of methods for identifying lncRNA-protein interactions[a)]

Method	RNA pulldown	RIP and RIP-chip/seq	ChIRP and CHART	CLIP
Mode	One RNA to many proteins	One protein to many RNA species	One RNA to many proteins and DNAs	One protein to many RNA species
Characteristics	A. Transcribed RNAs or synthetic RNAs *in vitro* for labeling. B. Capture RBPs with labeled RNA. C. Enrich endogenous, over-expressed, and *in vitro* translated protein.	A. Detect interaction of individual protein with specific RNA or RNA species. B. High-throughput finding of RNA species with microarray and high throughput sequencing. C. Detect RNAs by qRT-PCR.	A. Short complementary DNA oligonucleotide probes target RNA. B. High-throughput discovery of RNA-bound proteins and DNAs.	A. Crosslinking RNA and protein by photoreaction or chemical reaction. B. Capture RNA-protein interactions *in vivo*. C. High-throughput discovery of protein-bound RNAs.

a) RIP, RNA-binding protein immunoprecipitation; ChIRP, chromatin isolation by RNA purification; CHART, capture hybridization analysis of RNA targets; CLIP, crosslinking-immunopurification.

The basic mechanisms of RIP are very similar to ChIP. Pandey et al. [96] have found that Kcnq1ot1 interacts with both H3K9 methyltransferase and the PRC2 complex by RIP. Rinn et al. [9] performed RIP with an antibody against hnRNP-K, and validated the interaction between endogenous lincRNA-p21 and hnRNP-K in nuclear extracts. Native RIP previously identified Xist, Tsix, and RepA as PRC2-interacting ncRNAs [93]. RIP-Chip and RIP-seq involve RIP coupled with microarray and high-throughput sequencing, respectively. These techniques have been used to find interactions between one specific protein and a pool of RNA species [130,131]. Chromatin-modifying complexes, especially Polycomb proteins, play important roles in cell biology and human disease. Many lncRNAs interact with chromatin-modifying complexes and regulate gene expression. Ahmad et al. have used RIP-Chip to reveal that many lincRNAs interact with chromatin-modifying complexes [98]. Zhao et al. [131] developed the RIP-seq method and captured a genome-wide pool of >9000 RNAs interacting with PRC2 in ES cells.

3.3.3 *ChIRP and CHART*

Chromatin isolation by RNA purification (ChIRP) allows high-throughput discovery of RNA-bound proteins and DNA [132]. The RNA of interest is hybridized to biotinylated complementary oligonucleotides, and isolated using streptavidin beads. Co-purified chromatin is eluted for protein or DNA. These are identified in downstream assays, such as deep sequencing, which is termed ChIRP-seq. Chu et al. [132] have found that ChIRP-seq of three lncRNAs, *Drosophila* roX2, human TERC and HOTAIR, reveal that RNA occupancy sites in the genome are numerous, sequence-specific and focal. roX2 occupancy is enriched over the X chromosome, and increases tendency toward the 3′ end of each gene. In addition, motif analyses of the sequencing data have revealed a significantly enriched DNA motif that is identical to the male-specific lethal (MSL) motif. TERC ChIRP-seq has shown significant enrichment of telomeric DNA and Wnt receptor signaling pathway genes. HOTAIR genome-wide occupancy sites are enriched for genic regions, notably regions of promoters and introns. Focal sites of HOTAIR occupancy suggest that HOTAIR may nucleate Polycomb domains [132]. Simon et al. [133] have developed a method termed capture hybridization analysis of RNA targets (CHART) to determine the genomic binding sites of a specific ncRNA. CHART is a hybridization-based technique that is used to specifically enrich the protein and DNA targets of endogenous lncRNAs. This method is similar to ChIRP; however, the main difference lies in the design of probes to target lncRNA. They have been inspired by RNA FISH to design dozens of short complementary DNA oligonucleotide probes to tile the entire length of the lncRNA [134,135]. These short probes are called C-oligos. Simon et al. have adapted an RNaseH mapping assay to probe sites on lncRNA that are available to hybridization [133,136–138].

3.3.4 *CLIP*

Interaction between individual RNA-binding proteins or large complexes and different types of RNA is critical in gene regulation. The Crosslinking-immunopurification (CLIP) strategy is a breakthrough in the detection of RNA–protein interactions *in vivo* [139–141]. CLIP has three variants: high-throughput sequencing of CLIP (HITS-CLIP) [142,143], photoactivatable ribonucleoside-enhanced CLIP (PAR-CLIP) [144], and individual nucleotide resolution CLIP (iCLIP) [145]. In the CLIP procedure, whole tissues or individual cells are irradiated with ultraviolet light (UV), which generates covalent bonds between the RNA and protein when RNA–protein complexes are in close contact. Following UV-crosslinking, RNA-binding proteins (RBPs) can be purified under stringent conditions. The first CLIP experiment demonstrated NOVA-dependent splicing regulation [141]. HITS-CLIP has provided new insights into genome-wide NOVA binding sites in the brain [143]. Yeo and colleagues [142] have applied HITS-CLIP to study binding sites of FOX2 in hESCs, and found the most enriched hexamer was UGCAUG by motif analysis. Fink et al. [146] have performed a HITS-CLIP study of the yeast KHD1 protein, an RBP hypothesized to play a role in the development of diploid yeast. Application of CLIP has also uncovered ternary interactions among proteins, small RNAs and RNA. Ago HITS-CLIP analyses identified Ago-microRNA (miRNA) interactions, and revealed a footprint of Ago–mRNA binding sites. These two datasets were overlaid to decode which miRNAs bound to specific sites within mRNAs [147]. Another database, starBase (small non-coding RNAs target base), facilitates miRNA-target interaction maps from high-throughput data of CLIP-seq and degradome-seq [148]. Multiple RBPs, cooperatively modulating the stability, localization and modification of RNA, are critical parts of the cellular machinery that affect the regulative activity of these RNAs. CLIP-seq provides transcriptome-wide coverage for mapping RBP-binding sites. Khorshid et al. [149] have developed the CLIPZ database, which includes binding site data for multiple RBPs.

3.3.5 *Bioinformatics*

Bioinformatic prediction of the functions of lncRNAs gives helpful information for further exploration. This method avoids blindness of functional research and reduces experimental cost. Based on microarray expression profiles, Liao et al. [150] annotated the functions of about 340 lncRNAs. catRAPID (http://big.crg.cat/gene_function_and_evolution/services/catrapid), an online algorithm, mainly predicts associations of proteins and RNA [151]. This algorithm evaluates the interaction tendency between proteins and RNA, based on the secondary structure, hydrogen bonding and intermolecular force. Recent advances have shown that target RNAs can be post-transcriptionally regulated by com-

binatorial actions of RBPs and miRNAs. Anders et al. [152] have provided a database, doRiNA, that facilitates deciphering this post-transcription regulatory code.

4 Perspectives

Compared with protein-coding mRNA or small ncRNAs, studies into the functions of lncRNAs remain in infancy. (i) The definition of lncRNAs remains controversial. It is too arbitrary to define lncRNAs as a class of RNA molecule with more than 200 bases, because there are many ncRNAs with a length less than 200 bases that are neither small RNAs nor structural RNAs [1]. (ii) Elucidation of the biological functions of lncRNAs is difficult. lncRNAs are associated with gene regulation, and lncRNA deregulation has been shown to be involved in many diseases. Because of complications of the types and functions of lncRNAs, and poor conservation of lncRNAs between species, fully understanding the functions of lncRNAs remains difficult. (iii) Databases on lncRNAs are insufficient. (iv) Functional prediction tools for lncRNAs are few. (v) Research fields remain to be expanded. (vi) New technology for studying lncRNAs is inadequate. (vii) The potential applications of lncRNAs in the medical field are not clear.

With increasing attention on this field, the understanding of lncRNAs is gradually deepening. lncRNAs exert their biological functions on organisms through a variety of mechanisms, and their dysfunctions are related to the development of many diseases. To elucidate the more subtle regulatory roles of lncRNAs, technological advances are required in high-throughput imaging of RNAs, and high-resolution identification of proteins, RNA and DNA-binding partners of lncRNAs. This will allow further resolution of the functions of lncRNAs, their molecular mechanisms, and their pathological mechanisms in the development of diseases.

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