The nuclear functions of long noncoding RNAs come into focus

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ABSTRACT

Long noncoding RNAs (lncRNAs), defined as untranslated and tightly-regulated transcripts with a length exceeding 200 nt, are common outputs of the eukaryotic genome. It is becoming increasingly apparent that many lncRNAs likely serve as important regulators in a variety of biological processes. In particular, some of them accumulate in the nucleus and function in diverse nuclear events, including chromatin remodeling, transcriptional regulation, RNA processing, DNA damage repair, etc. Here, we unite recent progresses on the functions of nuclear lncRNAs and provide insights into the future research directions of this field.

1. Introduction

It was long assumed that only a small proportion of the eukaryotic genome is actively transcribed to generate templates for protein translation. However, RNA deep sequencing of eukaryotic transcriptomes has revealed that transcription is pervasively existing across eukaryotic genomes and a diverse group of RNA classes with little or no protein-coding capacity are produced [1,2]. Among these transcripts, long noncoding RNAs (lncRNAs) are ranging from 200 nt to 100 kb in length. Although most lncRNAs (e.g. X-inactive specific transcript, aka XIST) are generated with splicing signals and/or modifications similar to that of protein-coding genes as described by a comprehensive analysis [3], a number of lncRNAs are processed in unexpected ways. For example, the 3' end of MALAT1 (Metastasis associated lung adenocarcinoma transcript 1) and NEAT1 (Nuclear enriched abundant transcript 1) are processed by the tRNA biogenesis machinery, generating a stable triple helix structure instead of a poly(A) tail [4-6]. Circular RNAs are generated via back-splicing, a reaction by which a splicing donor is joined to an upstream acceptor [7-9]. Interestingly, some of lncRNAs are highly conserved at the sequence level. The expression levels of certain lncRNAs are higher than most protein-coding genes in a cell- and/or tissue-specific manner. Importantly, the dysregulation of many lncRNAs are associated with various human diseases, such as cancers and brain disorders. For example, H19 is one of the most abundant lncRNAs during fetal development in mammals [10,11]. H19 is robustly induced during embryogenesis at a level comparable to the house-keeping gene β-actin, but is downregulated significantly after birth. Nevertheless, it remains highly expressed in adult skeletal muscle and heart [10,11]. Loss of H19 induces precocious muscle differentiation and defective embryogenesis [10,11].

Similar to mRNAs, lots of lncRNAs display a post-transcriptional role in the cytoplasm as well summarized by Rashid et al. [12]. For example, cytoplasmic lncRNA-p21 has an inhibitory effect on the translation activity of CTNNB1 and JUNB by sterically blocking their transcripts [13]. In addition, circular RNA ND1 and ND5 can promote the mitochondrial entry of nuclear-encoded proteins by interacting with the mitochondrial importation complex [14]. But it is important to note that there are also many lncRNAs accumulating and functioning in the nucleus, which has gained a broad interest in recent years. These nuclear lncRNAs exert critical roles in chromatin remodeling, transcriptional regulation, RNA processing and modification, etc. In this review, we will discuss the functions and underlying mechanisms of several well-studied nuclear lncRNAs and provide insights into the future research directions of nuclear lncRNAs in the field.

2. Nuclear speckle-associated lncRNA MALAT1 (metastasis associated lung adenocarcinoma transcript 1)

The MALAT1 locus is located on human chromosome 11q13 and is able to generate a long non-coding and nuclear-retained transcript with a length of ~8 kb [15-17]. Despite being transcribed by RNA polymerase II (Pol II) [18,19], mature MALAT1 transcript does not contain a
MALAT1 accumulates in the nucleus, whereas *mascRNA* mainly localizes to the cytoplasm (Fig. 1A) [4]. Although MALAT1 lacks a poly(A) tail, it is highly abundant in human and mouse tissues [16,22,23] and expressed at a level higher than a number of housekeeping genes [24], indicating that the 3' end of MALAT1 is able to resist 3' degradation by 3'-5' exonucleases. Indeed, a following study found that the highly conserved A- and U-rich motifs at the 3' end of MALAT1 can form a triple helix via base-pairing, and disrupting the triple helix triggers the degradation of MALAT1 (Fig. 1B) [5,6,25], demonstrating that this extensive secondary structure is able to protect mature MALAT1 from 3'-5' exonucleolytic decay. Different from *mascRNA* or many other ncRNAs, MALAT1 specifies localizes in nuclear speckles (also called as interchromatin granule cluster) [23,26,27], nuclear domains that are composed of multiple factors involved in transcription activation or repression, pre-mRNA splicing, mRNA modification, and mRNA nuclear export [28-30]. Three nuclear speckle proteins (RNPS1, IBP160, and SRm160) and two distinct motifs on MALAT1 contribute to the specific localization [27]. Such localization suggests that MALAT1 may play important roles in some molecular processes in the nucleus. In fact, MALAT1 is not a structural RNA required for the maintenance of nuclear speckle integrity [31,32], but functions in chromosome segregation during mitosis [32]. Lack of MALAT1 results in aberrant mitosis and the fragmentation of cell nuclei [32]. Intriguingly, Tripathi and colleagues found that MALAT1 interacts with the serine/arginine (SR) splicing factors, controls the distribution and phosphorylation status of SR proteins in nuclear speckles and, in turn, modulates alternative splicing (AS) events of a set of SR-regulated pre-mRNAs in HeLa cells (Fig. 1C) [32]. It is thus likely that the cycling of at least some of SR proteins between speckle domains and sites of transcription is regulated by MALAT1. However, there are several contradictory observations that MALAT1 had almost no effect on the localization of nuclear speckle components or phosphorylation status of SR proteins in knockout mice [24,33,34]. Besides, no obvious connection between MALAT1 and AS regulation was found [24,33]. These inconsistent results might be explained by the possibility that MALAT1 has evolved a function in human cells that is not easily detected in mice or the function of MALAT1 is simply cell/tissue-specific, since MALAT1 expression varies significantly among tissues and cells [16,33].

MALAT1 has also been reported to participate in modulation of transcription (Fig. 1C). Indeed, a comprehensive analysis of genomic binding sites of MALAT1 implied that it exhibits localization to many actively transcribed chromatin sites [35]. The human *LTBP3* is a gene downstream of the MALAT1 locus and has an opposite transcription orientation to that of MALAT1. MALAT1 activates the transcription of *LTBP3* by promoting the recruitment of the key transcription factor Sp1 to the *LTBP3* promoter in mesenchymal stem cells [36]. A large reduction in expression of *NEAT1*, an IncRNA locus upstream of MALAT1, was observed in MEFs obtained from MALAT1 knockout mice [27]. In addition to the cis effect on neighboring genes, the localization of the polycomb 2 protein (Pc2), a key histone modifier, is controlled by MALAT1, which in turn influences expression of many genes [37]. Yang and colleagues demonstrated that the association between MALAT1 and
unmethylated Pc2 promotes E2F1 SUMOylation, resulting in a biochemical switch of the Pc2 chromodomain from interacting with repressive histone marks to preferring active histone marks. Subsequently, a number of growth-control genes are activated by the event [37].

MALAT1 can also act as a microRNA sponge in the nucleus in an AGO2-dependent manner (Fig. 1C) [38-41]. For example, there are two targeting sites for microRNA-9 on MALAT1 transcript. The MALAT1 level was significantly reduced in AGO2 immunoprecipitates purified from cells treated with anti-microRNA-9 as compared to the control sample [38]. In another case, MALAT1 can modulate c-Myc expression by sponging miRicoRNA-34a in melanoma cells [40].

MALAT1 transcript was first discovered in a screening for genes whose expression have a potential relationship to metastasis in early-stage non-small cell lung cancer [16]. Afterwards, MALAT1 was found to be evolutionally conserved from mouse to human and widely expressed in numerous cancer tissues [42-45], implying that the dysregulation of MALAT1 plays crucial roles in cancers and MALAT1 can be used as a valid cancer biomarker as well as a therapeutic target for clinical application (Fig. 1C). For instance, MALAT1 have been found as an important regulator of lung cancer metastasis probably due to its effect on metastasis-associated gene expression [46]. Interestingly, the MALAT1 ASO (antisense oligonucleotide) treated mice was able to resist to EBC-1 tumor metastasis to the lung, indicating a potential application of ASOs against MALAT1 in preventing lung cancer from spreading [46]. In colorectal cancer (CRC), the binding of MALAT1 to the tumor suppressor PSF (PTB-associated splicing factor) dissociates PTB (poly-pyrimidine-tract-binding protein) from PSF. The released PTB promotes CRC cell proliferation and tumor growth [47]. Two recent studies indicated that the secondary structure of MALAT1 varies in different cancers [48,49]. By dimethyl sulfate-sequencing and RNA structure analysis, MALAT1 is likely to be more unstructured in chronic myeloid leukemia, but undergoes 18 novel structural rearrangements in cervical cancer [49]. This provides new insights into how RNA structure affects its regulatory role under various conditions. Besides cancers, the dysregulation of MALAT1 is also involved in many other physiological processes and pathological diseases, including brain development [26,50], vascular growth [51], cardiovascular diseases [52,53], neurological disorders [54-57], just to name a few. Unfortunately, most of the precise mechanisms underlying the MALAT1-associated diseases still remain unclear. Therefore, further studies are needed to clarify these questions.

3. Nuclear paraspeckle-associated lncRNA NEAT1 (nuclear enriched abundant transcript 1)

NEAT1 is originally identified as a large and infrequently spliced transcript in a genome-wide screening to search for nuclear ncRNAs that might modulate gene expression [23]. The NEAT1 locus is adjacent to the MALAT1 locus on human chromosome 11q13 and generates two overlapping isoforms [58,59]. NEAT1-1, aka MENε, is the short isoform with a length of ~3700 nucleotides [60]. The 3’ end of NEAT1-1 undergoes the canonical cleavage/polyadenylation steps and, in turn, terminates in a poly(A) tail (Fig. 2A). On the contrary, the long version of NEAT1 (NEAT1-2, aka MENβ) is over 23 kb and stabilized by a triple helix structure following RNase P cleavage at its 3’ end (Fig. 2A and B) [5,6,60], which is similar to MALAT1. Although none of the two isoforms has an open reading frame or a capacity to yield a peptide, they are highly and ubiquitously expressed in a wide range of tissues, such as ovary, prostate, colon, and pancreas [23]. As a type of architectural lncRNA, NEAT1 is essential for organization and integrity of nuclear paraspeckles [61-64], irregularly shaped subnuclear bodies closed to the NEAT1 transcription site [31]. NEAT1 translocates immediately to
nuclear paraspeckles once it is transcribed by RNA Pol II [65]. During daughter nuclei reformation, NEAT1 transcription happens prior to the accumulation of the paraspeckle-associated proteins PSP1/p54 in paraspeckles [31]. NEAT1 depletion leads to loss of paraspeckles, whereas overexpression of NEAT1 increases paraspeckle number [31,60,65,66], implying that the paraspeckle-associated proteins might be multifunctional when endogenous NEAT1 expression is altered. In fact, NEAT1 has been found to bind specifically to PSP1/p54 [31]. Interestingly, PSP1 forms a heterodimer with p54 in a NEAT1-independent manner [66]. But PSP1 without the RNA recognition motifs no longer exhibits an interaction with NEAT1 or paraspeckles [31]. Furthermore, NEAT1 depletion has no significant effect on the protein level of PSP1 or p54, excluding the possibility that NEAT1 regulates the degradation of some paraspeckle proteins [60]. Therefore, nuclear paraspeckle is built on NEAT1 and the dysfunction of nuclear paraspeckle could be triggered by NEAT1 depletion. For example, the nuclear export of transcripts containing paraspeckle-retention elements (Alu repeats in humans and SINEs in mouse) or hyper-edited A-to-I is enhanced by NEAT1 depletion [66,67].

NEAT1 not only functions as an architectural component of paraspeckles, but also is related to some other organelles (Fig. 2C). For example, NEAT1 and paraspeckles are crucial for mitochondrial homeostasis [68,69]. A recent study demonstrated that loss of mitochondrial proteins or mitochondrial stress results in abnormal expression of NEAT1 as well as a remarkable increase of elongated nuclear paraspeckles [68]. Moreover, many mRNAs of nuclear-encoded mitochondrial proteins contain paraspeckle-retention elements at their 3’ UTRs and is associated with NEAT1 [68], indicating a strong regulatory communication between nucleus and mitochondria. Abnormal NEAT1 expression leads to mitochondrial dysfunction, such as altered mitochondrial respiration and ATP production rate [68,69].

NEAT1 has strong regulatory effects on many molecular processes (Fig. 2C). Jiang and colleagues found that NEAT1 and its binding partner PSP1/p54 heterodimer can enhance pri-miRNA processing [70]. NEAT1 may provide a binding platform for various pri-miRNAs and microprocessor components that are attracted by the pri-miRNA-like hairpin structures of NEAT1 [70]. Additionally, NEAT1 can regulate the splicing of PPAR2 (peroxisome proliferator activated receptor) pre-mRNA temporally via the SR protein SRp40 mediated pathway during adipogenesis. In this case, NEAT1 binds to SRp40 and potentiates its phosphorylation status by Clk kinase [71]. Wang and colleagues found that NEAT1 can activate viral gene expression by recruiting STAT3 (signal transducer and activator of transcription 3) to their promoters in response to herpes simplex virus-1 (HSV-1) infection [72]. Similarly, their following study showed that NEAT1 recruits the acetyltransferase CBP/P300 complex to the promoters of the endocytosis-related genes, thereby altering H3K27 acetylation and H3K27 crotonylation in Alzheimer disease [73].

Except for RNA processing or transcriptional regulation, several studies have suggested that NEAT1 has a profound connection with translation activation or protein stability control. For example, during RNA polymerase I inhibition, NEAT1-sequestered p54 and PSF are released from nuclear paraspeckles upon NEAT1 depletion and relocated to c-Myc mRNAs for its translation activation [74]. Curiously, NEAT1 depletion has almost no effect on the protein level of c-Myc under normal conditions [74], indicating that the NEAT1-mediated c-Myc translation only occurs in stressed conditions. In CD4+ T cells, NEAT1 physically binds to STAT3 protein and keeps it from ubiquitination [75]. This was exemplified by the NEAT1 knockdown experiment which led to a large reduction in ubiquitination labeled STAT3 protein [75]. Likewise, the half-life of another NEAT1-binding protein DDx5 is drastically increased upon overexpression of NEAT1 in HT29 cells [76].

Similar to many other ncRNAs, NEAT1 consists of potential targeting sites for various microRNAs [77–82]. Importantly, plenty of these microRNA-target genes are oncogenes and contribute to tumorigenesis. Disrupting the cross-talk between NEAT1, microRNAs, and microRNA-target genes may promote proliferation, migration, invasion of cancer cells. Thus NEAT1 could be an effective target for the diagnosis and therapy of many cancer types. In prostate cancer, NEAT1 modulates the expression of the oncogene HMGA2 by sponging mircoRNA-98-5p [81]. In gastric cancer, the binding of microRNA-365a-3p to NEAT1 enhances ABC44 expression [80]. In esophageal Squamous Cell Carcinoma, mircoRNA-129/CTBP2 axis is controlled by NEAT1 [82]. Nevertheless, it is still elusive how and why cytoplasmic microRNAs are controlled by a nuclear IncRNA.

An increasing number of studies focusing on NEAT1’s function in physiology have proved that the intensive upregulation of NEAT1 in several human diseases is associated with a drastic decrease in patient survival (Fig. 2C) [83–87]. In regard to Parkinson’s disease (PD), a chronic and progressive brain disorder that affects movement, NEAT1...
expression was found to be significantly upregulated in PD mice [83,84]. Interestingly, depletion of NEAT1 could reverse apoptosis induced by PD inducers possibly by controlling the expression of some PD contributors, such as α-synuclein and PINK1 [83,84]. Regarding non-alcoholic fatty liver disease, loss of NEAT1 inhibits the expression of acetyl-CoA carboxylase and fatty acid synthase through the mTOR/S6K1 signaling pathway, implying that NEAT1 acts as a potent regulator in the biosynthesis of lipid and insulin signaling transduction [88]. With intensive researches of NEAT1-related diseases, we believe that a therapeutic application of NEAT1 is coming soon.

4. Chromatin-associated lncRNA XIST (X-inactive specific transcript)

Less than 3% of the ancestral genes survive on the male Y chromosome, while X chromosome retains more than 97% [89,90]. Therefore, males and females have unbalanced genetic content in mammals. To minimize sex chromosome-linked gene expression imbalance between XX females and XY males, a randomly chosen X chromosome is transcriptionally silenced in early female embryogenesis. This dosage compensation process is called X chromosome inactivation (XCI) and is initiated by the IncRNA XIST [91-96]. It is important to note that XCI by no means equal to loss of X chromosome, since 3%-20% of the X-linked genes from the inactive X (Xi) chromosome are still activated and indispensable for female embryogenesis. This is exemplified by Turner syndrome, a genetic disorder disease only occurs in females with only a single X chromosome [97,98]. XIST was first described as a long non-coding transcripts generated exclusively from the Xi chromosome in the early 1990s [99-103]. Once generated, XIST spreads across the entire length of the Xi chromosome, resulting in gene repression chromosome-widely. Further sequence analyses revealed that XIST is a 15-17 kb spliced and polyadenylated RNA which contains blocks of local tandem repeat elements designated A-F (Fig. 3) [102,103]. Significant progresses have elucidated the functional roles of each individual repeat in establishment of X chromosome-linked gene silencing. Hence, we will focus our discussion on these functional elements as well as their RBPs (RNA-binding proteins) in this section.

Using a series of inducible XIST transgenes, Wutz and colleagues found that XIST mutations that lack A-repeat, consisting of 7-8 copies of a 24 nt GC-rich core sequence at the 5’ end of XIST, abolish silencing activity and lead to a decrease in stability without influencing localization [104]. Furthermore, the transcription activity of XIST appears to be affected by deletion of the endogenous A-repeat region in DNA [105]. Besides affecting XIST itself, the role of A-repeat in chromosome silencing is also linked to its binding proteins. For example, A-repeat contributes to the interaction with RBM15 (RNA binding motif protein 15) which further targets the methyltransferase METTL3/14 to specific RNAs, including XIST, for addition of N6-methyladenosine (m6A modification) [106,107]. Importantly, loss of the m^6^A catalytic subunit or the m^6^A reader leads to a major silencing deficiency, indicating a role of m^6^A in XIST-mediated chromosome silencing [106]. Contradictorily, only a minor decrease in X silencing efficiency was observed in either RBM15 knockdown or knockout cells [106,108]. The phenomena might be explained by the finding of RBM15B, the RBM15 homolog, which shares redundant functions with RBM15 [106,109]. SPEN is another interactor of A-repeat [110]. Depletion of SPEN results in impaired silencing of the X chromosome-linked genes, such as Fbx17, without disrupting XIST localization [107,108,110]. In addition, SPEN might initiate transcriptional repression by actively recruiting the transcriptional co-repressor complex and the histone deacetylase HDAC3 [112-114]. Unlike RBM15 and SPEN, LBR (Lamin B receptor) has a widespread distribution across XIST [115,116]. The major binding site encompasses the entire A- and F-repeat as proved by LBR CLIP-seq analysis [115,116]. Losing the interaction between XIST and LBR leads to impaired chromosome silencing [108,116]. B- and C-repeat have been reported to be critical for XIST spreading as well as the recruitment of hnRNP K (heterogeneous nuclear ribonucleoprotein K) and the polycym silencing complexes to XIST [110,117,118]. Chu and colleagues found that defective chromosome silencing can be induced by depletion of hnRNP K [110]. With further ICoFISH (combined immunofluorescence and RNA fluorescence in situ hybridization) experiments, they demonstrated that hnRNP K colocalizes with H2AK119ub and H3K27me3, the earliest chromatin modifications occurring in XCI [110]. Depletion of hnRNP K reduces the accumulation of H3K27me3 and H2AK119ub along Xi without influencing the global level of these modifications, suggesting Xi undergoes a specific modification. Indeed, hnRNP K depletion specifically dissociates XIST from the polycym silencing complexes [110]. C-repeat, together with E-repeat, is also responsible for the binding of hnRNP U (heterogeneous nuclear ribonucleoprotein U) to XIST and, in turn, XIST localization to chromatin [108,110,119-121]. But whether correct XIST localization depends on a combination of additional factors is still elusive, since the effect of hnRNP U cannot be confirmed in certain cell types [122,123]. In fact, an interesting study demonstrated that YY1 protein, capable of binding both RNA and DNA, acts as an adaptor in docking C-repeat of the RNA onto the F-repeat region of the XIST locus [124]. By deleting D-repeat through CRISPR/Cas9 technique in HEK293T cells, a significantly reduction in XIST expression was observed, implying that D-repeat might play an important role in the stability or transcriptional activity of XIST [125]. Consequently, the silencing ability of XIST was abrogated as proved by the phenomenon that levels of some X chromosome-linked genes were upregulated [125].

3D structural reorganization is another critical pathway for establishment of Xi gene repression by XIST, together with SmcHD1 (Structural maintenance of chromosomes flexible hinge domain containing 1) and the polycym factors [126-134]. In this mechanism, the X inactive genes are relocated from the peripheral to the interior of chromatin during XCI, thereby being isolated from RNA Pol II and other general transcription factors [126].

XCI is not only a dosage compensation process happening during early female embryogenesis and related to X-linked diseases in females, but also a critical modifier of many other diseases including cancers and neurological disorders. For example, XIST could affect cancer development and progression by sponging various microRNAs, thereby influencing the expression of some oncogenes [135-137]. On the other hand, Yue and colleagues found that the level of XIST is significantly increased in AD (Alzheimer’s disease) mice models, suggesting that XIST might be used as a diagnostic marker for AD [138].

5. Nuclear circular RNA

Protein-coding genes of the eukaryotic genome are typically split by one or multiple introns. During the production of a mature mRNA, introns must be removed and exons are joined together in a linear order, which is known as canonical splicing [139-141]. Alternatively, splicing events can also occurs in a way that a splicing donor is joined to an upstream acceptor, thereby generating a covalently-closed RNA (circular RNA) [7,142-145]. Despite the first circular RNA being discovered in 1976 [146], only recently has it been appreciated that these covalently-closed molecules represent a common class of transcriptional outputs from numerous protein-coding genes in the eukaryotic system [147,148]. So far, many labs have been putting a lot of efforts into characterization of circular RNA biology, and thus obtained remarkable progresses as well reviewed in many papers [7-9,143,149-154]. In short, the biogenesis of circular RNA is initiated by base-pairing between the flanking intronic repeats as well as some RBPs, including QKI, Mbl, ADAR1, RBM20, FUS, etc [155-159]. The degradation of circular RNA is controlled by different exonucleases and the platform protein gawk (aka GW182) in various context [160-164]. Once generated, most circular RNAs are exported to the cytoplasm in a length-dependent manner with the assistance of the DExH/D-box helicase DDX39A and DDX39B [165,166]; however, a small amount of circular RNAs accumulate in the
Fig. 4. The biogenesis of circular RNAs. Most circular RNAs are generated from protein-coding genes via back-splicing by which a splicing donor is joined to an upstream acceptor. In other cases, circular RNAs are derived from lariat intronic RNAs that escape from debranching.

Table 1

| circRNA          | Type   | Molecular Mechanism                                                                 | Ref  |
|------------------|--------|--------------------------------------------------------------------------------------|------|
| circeIF3J        | EicRNA | Interacts with RNA Pol II and U1 snRNP to regulate transcription of its host gene    | [167]|
| circpAIP2        | EicRNA | Interacts with RNA Pol II and U1 snRNP to regulate transcription of its host gene    | [167]|
| circANKRD52      | ciRNA  | Interacts with RNA Pol II to regulate transcription of its host gene                  | [168]|
| circln2-INS      | ciRNA  | Interacts with TDP-43 to regulate transcription                                       | [169]|
| circFECR1        | EicRNA | Induces promoter hypomethylation with the demethylase TET1                           | [170]|
| circ2082         | EicRNA | Promotes DICER nuclear translocation via RBM3                                         | [171]|
| cia-cgAS         | EicRNA | Inhibits cGAS as a decay                                                              | [172]|
| circSEP          | EicRNA | Regulates alternative splicing of its host gene via R-loop                            | [173]|
| circCRM1         | Intergenic ciRNA | Maintains normal centromeric chromatin organization via R-loop | [174]|
| circSMARCA5      | EicRNA | Downregulates its host gene expression via R-loop                                     | [177]|

nucleus. Here, we will focus our discussion on nuclear circular RNAs and their functions in this section.

In searching for lncRNAs involved in transcription regulation, Li and colleagues identified a special subclass of Pol II-binding circular RNA, also called EicRNAs (exon-intronic circular RNA), whose intron(s) between circularized exons are not spliced out (Fig. 4) [167]. Similar to most EicRNAs (exonic circular RNAs), EicRNAs are generated via back-splicing reactions in which the flanking Alu repeats facilitate their circularization [167]. Functionally, EicRNAs have cis regulatory effects on their host gene transcription activity (Table 1), since depletion of EicRNAs leads to a significant reduction in the levels of their nascent mRNAs. Further mechanism analyses indicated that EicRNAs interact with U1 snRNP directly at the promoter of their host gene. Loss of U1 snRNP or disrupting the U1 snRNP-EicRNA interaction by antisense morpholino (AMO) impairs the transcription-enhancing effect of EicRNAs [167]. Notably, EicRNAs not only localize to their sites of transcription, but also accumulate to other nuclear subdomains, suggesting other potential regulatory roles and mechanisms of EicRNAs [167]. Another example of nuclear intron-containing circular RNA is ciRNA (intronic circular RNA), a type of lariat RNA which escapes from debranching (Fig. 4) [168]. Like EicRNAs, ciRNAs (e.g. circANKRD52) are able to interact with the Pol II complex and modulate transcription efficiency of their host genes in cis (Table 1). Mechanistically, depletion of circANKRD52 has almost no effect on Pol II engagement, suggesting that circANKRD52 affects transcriptional rate but not transcription termination [168]. In another case, ciRNAs (circln2 in rat and circln5 in human), derived from the insulin gene, regulate transcription of genes involved in calcium signaling and insulin exocytosis via the RBP TAR DNA-binding protein 43 kDa (TDP43) in the nucleus (Table 1) [169]. Low expression of circln2 or circln5 leads to defective insulin secretion of β-cell to and are associated with type 2 diabetes [169]. EciRNAs are also capable of regulating transcription in the nucleus as exemplified by circFECR1. CircFECR1 can activate its host gene expression by inducing promotor hypomethylation with the demethylase TET1 (Table 1) [170].

Besides transcription, some nuclear circular RNAs can affect the function of their RBPs. For example, a recent study demonstrated that circ2082 has an important role in nucleocytoplasmic distribution of the endoribonuclease DICER, a key component of pre-microRNA processor complex (Table 1) [171]. In this case, circ2082 is recruited to DICER by RNA binding motif protein 3 (RBM3) in the nucleus of glioblastoma cell. The interaction is crucial for nuclear retention of DICER, since depletion of circ2082 results in normalization of DICER localization to the cytoplasm [171]. The change in nucleocytoplasmic distribution of DICER further reestablishes the assortment of cellular microRNAs (microRNAome) [171]. Because the dysregulation of microRNAome is associated with cancers, circ2082 could be used as a therapeutic target for cancer treatment. Circular RNA cia-cgAS acts as a protein decoy for the DNA sensor cGAS and inactivates its synthase activity under homeostatic conditions, which in turn inhibits cGAS-mediated autoimmune responses (Table 1) [172].

Interestingly, certain nuclear circular RNAs are able to form an RNA:DNA hybrid (R-loop) with the genomic DNA. In Arabidopsis, nuclear circSEP derived from exon 6 of the SEPALLATA3 gene was reported to affect the splicing of its host gene through an R-loop mechanism (Table 1). Formation of the R-loop leads to an increased level of a linear SEP transcript isoform which lacks exon 6, in turn driving floral homeotic phenotypes [173]. Importantly, circSEP:DNA R-loops are more abundant than their linear RNA:DNA counterparts, indicating the specific role of circSEP in R-loop formation [173]. In Zea mays, circular RNA:DNA R-loops are integral components of centromeric chromatin [174]. The centromeric retrotransposons derived circular RNAs were found to bind to the centromere through R-loops (Table 1). Disrupting the R-loop results in abnormal localization of the centromeric H3 variant (CENH3), in turn affecting centromeric chromatin organization [174]. The centromeric retrotransposons derived circular RNAs were found to bind to the centromere through R-loops (Table 1). Disrupting the R-loop results in abnormal localization of the centromeric H3 variant (CENH3), in turn affecting centromeric chromatin organization [174]. SMARCA5 is a key component of DNA damage repair pathway and is required for chromatin remodeling [175,176]. In breast cancer cells, circSMARCA5 can downregulate the expression of the SMARCA5 gene via R-loop (Table 1). Therefore, circSMARCA5 has a negative effect on DNA repair capacity [177].

6. Concluding remarks

Eukaryotic nucleus is a membrane-bound organelle contains most genetic information and controls gene expression. Many molecular processes, such as transcription, splicing, replication, histone modification, and DNA repair, occur in the nucleus and are regulated combinatorially by various protein factors. Only until recent 20 years, lncRNAs have been considered important regulators in the processes mentioned above [19,178–180]. Although some of lncRNAs (e.g. XIST) have general effects on nuclear events, the majority of them seem to act uniquely on certain genes, implying that the total numbers of functional
nuclear lncRNAs (annotated or unannotated) may be underestimated. Thus defining the novel nuclear roles of lncRNAs will help us understand the complex network of regulation.

Having established an association between a lncRNA and a nuclear event, we could reason a possible mechanism based on the phenotype. Informed by the studies discussed in above sections, we will summarize lncRNA biology is just coming.

To identify the mechanisms lncRNAs exert, further efforts need to be made. First, lncRNAs might indirectly affect certain events via controlling the expression or subcellular localization of the key protein factors. With the development of high-throughput RNA-seq technique and single molecule tracking system, this possibility could be easily examined. Second, lncRNAs might act as a molecular platform, bringing diverse proteins together into an RNP complex, or a decoy by sponging RNAs or even proteins and preventing them from associating with their targets. In these two cases, deletion analyses can be performed. If the hypothesis is right, then deletion of a protein binding motif in lncRNAs or a RNA binding motif in proteins leads to loss of function. In addition, a variety of high-throughput sequencing methods were developed to identify the interactionomes of nuclear lncRNAs in vivo (Table 2). These tools could help us map RNA-DNA [181–189], RNA-protein [190–193], and RNA-RNA interactions [194–198]. Third, lncRNAs might be an organizer of nuclear architecture. Knockdown or knockout of these lncRNAs results in nuclear subdomain breakdown, thereby affecting unique reactions. Fourth, the act of transcription through a lncRNA locus seems to be regulatory, rather than the lncRNA product as discussed by Long et al. and Bassett et al. [178,179]. To answer the question, the lncRNA region in genomic DNA can be replaced by a random sequence without changing its promoter. Combinatorially, the lncRNA transcript can be knockdown by siRNA, ASO, or CRISPR-Cas13a system or functionally blocked by AMO.

In conclusion, the last 20 years have revealed that lncRNAs are not simply rare transcriptional noise, but pervasively exist in the eukaryotic system. Although numerous surprises have been disclosed, they only represent a glimpse of the tip of lncRNA biology. More efforts are needed to examine the 100 Talent Program of Chongqing University (030400110433). We thank the members of the Shan lab for Overseas Returned Scholars of Chongqing, China (cx2019142), the Natural Science Foundation of Chongqing (cx2019142), the National Science Foundation of China (32070633), the Ministry of Education of China (030400110433). We thank the members of the Shan lab for Overseas Returned Scholars of Chongqing, China (cx2019142), the Natural Science Foundation of Chongqing (cx2019142), the Ministry of Education of China (32070633), the Non-coding RNA Research 6 (2021) 70–79.

Table 2 The methods used to study RNA interactions.

| Method  | Description | Experimental Purpose | Enhanced Version | Ref |
|---------|-------------|----------------------|------------------|-----|
| CHIRP-  | A method to pull down and map the RNA-associated chromatin using biotinylated antisense oligos | Identification of RNA-DNA interaction | CHART-seq, CHIRT-seq, hicHCRP-seq | [181–185] |
| seq     |             |                      |                  |     |
| R-CHIP- | A method to pull down and map R-loops using catalytic-dead RNase H | Identification of R-loop | hicHCRP-seq | [186] |
| seq     |             |                      |                  |     |
| DRIP-seq| A method to pull down and map R-loops using 59.6 antibody | Identification of R-loop | bidDRIP-seq, DRIP-seq | [187–189] |
| seq     |             |                      |                  |     |
| CLIP-seq| A method to pull down and map the protein-binding RNA by immunoprecipitation | Identification of RNA-protein interaction | PAR-CLIP-seq, iCLIP-seq, hicLIP-seq | [190–193] |
| SHAPE-  | A method to predict RNA secondary structures using chemical modifiers | Prediction of RNA-RNA interaction | iSHAPE-seq, DMS-seq, SHAPE-seq2.0 | [194–197] |
| seq     |             |                      |                  |     |
| Frag-seq| A method to predict RNA secondary structures using endonuclease P1 | Prediction of RNA-RNA interaction | hicLIP-seq | [198] |

References

[1] P. Kapranov, A.T. Willingham, T.R. Gingeras, Genome-wide transcription and the implications for genomic organization, Nat. Rev. Genet. 8 (6) (2007) 413–423.
[2] M.B. Clark, et al., The reality of pervasive transcription, PLoS Biol. 9 (7) (2011), e1000625 discussion e1001102.
[3] T. Derrien, et al., The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression, Genome Res. 22 (9) (2012) 1775–1789.
[4] J.E. Wilusz, S.M. Freier, D.L. Spector, 3′ end processing of a long nuclear-retained noncoding RNA yields a rRNA-like cytoplasmic RNA, Cell 135 (5) (2008) 919–922.
[5] J.E. Wilusz, et al., A triple helix stabilizes the 3′ ends of long noncoding RNAs that lack poly(A) tails, Genes Dev. 26 (21) (2012) 2392–2407.
[6] J.A. Brown, et al., Formation of triple-helical structures by the 3′-end sequences of MALAT1 and MENbeta noncoding RNAs, Proc. Natl. Acad. Sci. U. S. A. 109 (47) (2012) 19202–19207.
[7] L. Chen, et al., Circular RNAs in eukaryotic cells, Curr. Genom. 15 (5) (2014) 312–318.
[8] J.E. Wilusz, A 360 degrees view of circular RNAs: from biogenesis to functions, Wiley Interdiscip. Rev. RNA 9 (4) (2018) e1478.
[9] M.S. Xiao, Y. Ai, J.E. Wilusz, Biogenesis and functions of circular RNAs come into focus, Trends Cell Biol. 20 (3) (2020) 226–240.
[10] A. Gabory, et al., The H19 gene: regulation and function of a non-coding RNA, Cytogenet. Genome Res. 113 (1–4) (2006) 188–193.
[11] A. Gabory, H. Jammes, L. Dandolo, The H19 locus: role of an imprinted non-coding RNA in growth and development, Bioessays 32 (6) (2010) 473–480.
[12] F. Rashid, A. Shah, G. Shan, Long non-coding RNAs in the cytoplasm, Dev. Reprod. Biol. 14 (2) (2016) 73–80.
[13] H. Yoon, et al., LINcRNA-p21 suppresses target mRNA translation, Mol. Cell 47 (4) (2012) 648–655.
[14] X. Liu, et al., Identification of meccRNRs and their roles in the mitochondrial entry of proteins, Sci. China Life Sci. 63 (10) (2020) 1429–1449.
[15] R. Lin, et al., A large noncoding RNA is a marker for marine hepatocellular carcinomas and a spectrum of human carcinomas, Oncogene 26 (6) (2007) 851–858.
[16] P. Ji, et al., MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer, Oncogene 22 (39) (2003) 8031–8041.
[17] X. Zhang, M.H. Hamblin, K.J. Yin, The long noncoding RNA Malat1: its physiological and pathophysiological functions, RNA Biol. 14 (12) (2017) 1705–1714.
[18] S. Djebari, et al., Landscape of transcription in human cells, Nature 489 (7414) (2012) 101–108.
[19] B. Yu, G. Shan, Functions of long noncoding RNAs in the nucleus, Nucleus 7 (2) (2016) 155–166.
[20] J.E. Wilusz, D.L. Spector, An unexpected ending: noncanonical 3′ end processing mechanisms, RNA 16 (2) (2010) 259–266.
[21] J.E. Wilusz, Long noncoding RNAs: Re-writing dogmas of RNA processing and stability, Biochim. Biophys. Acta 1859 (1) (2016) 128–138.
[22] K. Tano, et al., MALAT-1 enhances cell motility of lung adenocarcinoma cells by influencing the expression of motility-related genes, FEBS Lett. 584 (22) (2010) 6575–6580.
[23] J.N. Hutchinson, et al., A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains, BMC Genom. 8 (2007) 39.
[24] B. Zhang, et al., The IncRNA Malat1 is dispensable for mouse development but its transcription plays a cis-regulatory role in the adult, Cell Rep. 2 (1) (2012) 111–123.
[25] J.A. Brown, et al., Methyltransferase-like protein 16 binds the 3′-terminal triple helix of MALAT1 long noncoding RNA, Proc. Natl. Acad. Sci. U. S. A. 113 (49) (2016) 14013–14018.
[26] D. Bernard, et al., A long nuclear-retained non-coding RNA regulates synaptogenesis by modulating gene expression, EMBO J. 29 (18) (2010) 2382–2392.

[27] R. Miyagawa, et al., Identification of cis- and trans-acting factors involved in the localization of MALAT-1 noncoding RNA to nuclear speckles, RNA 18 (4) (2012) 738–751.

[28] D.L. Spector, A.I. Lamond, Nuclear speckles, Cold Spring Harb. Perspect. Biol. 3 (2) (2011).

[29] A.I. Lamond, D.L. Spector, Nuclear speckles: a model for nuclear organelles, Nat. Rev. Mol. Cell Biol. 11 (10) (2010) 605–612.

[30] L. Galganski, M.O. Urbanek, W.J. Krzyzosiak, Nuclear speckles: molecular organization, biological function and role in disease, Nucleic Acids Res. 45 (18) (2017) 10350–10368.

[31] C.M. Gerson, et al., An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles, Mol. Cell 33 (6) (2009) 717–726.

[32] V. Tripathi, et al., The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation, Mol. Cell 39 (6) (2010) 925–938.

[33] S. Nakagawa, et al., MALAT1 is not an essential component of nuclear speckles in mice, RNA 18 (8) (2012) 1487–1499.

[34] M. Eissmann, et al., Loss of the abundant nuclear non-coding RNA MALAT1 is compatible with life and development, RNA Biol. 9 (8) (2012) 1076–1087.

[35] J.A. West, et al., The long noncoding RNAs NEAT1 and MALAT1 bind active chromatin sites, Mol. Cell 55 (5) (2014) 791–802.

[36] B. Li, et al., Activation of LTR3 gene by a long noncoding RNA (IncRNA) MALAT1 transcript in mesenchymal stem cells from multiple myeloma, J. Biol. Chem. 289 (42) (2014) 29365–29375.

[37] L. Yang, et al., ncRNA- and P2c2 methylation-dependent gene relocation between nuclear structures mediates gene activation programs, Cell 147 (4) (2011) 773–788.

[38] E. Leucci, et al., microRNA-9 targets the long non-coding RNA MALAT1 for degradation in the nucleus, Sci. Rep. 3 (2013) 2535.

[39] M. Zhang, et al., MALAT1 sponges miR-34a and promotes the invasion and metastasis of colorectal cancer via SLAIN2 enhanced microtubule mobility, Elife Biology 41 (2019) 286–298.

[40] F. Li, et al., MALAT1 regulates miR-34a expression in melanoma cells, Cell Death Differ. 26 (6) (2019) 886–899.

[41] F. Tao, et al., miR-211 sponges IncRNA MALAT1 to suppress tumor growth and progression through inhibiting PI3K/Akt in ovarian cancer, FASEB J. (2018), f20180904950R.

[42] M. Zhao, et al., MALAT1: a long non-coding RNA highly associated with human cancers, Oncol. Lett. 16 (1) (2018) 19–26.

[43] Z.K. Li, et al., MALAT1: a potential biomarker in cancer, Canc. Manag. Res. 10 (2018) 6757–6768.

[44] A. Bhan, M. Soleimani, S.S. Mandal, Long noncoding RNA and cancer: a new paradigm, Canc. Res. 77 (15) (2017) 3965–3981.

[45] A. Sanchez Calle, et al., Emerging roles of long non-coding RNA in cancer, Canc. Sci. 109 (7) (2018) 2093–2100.

[46] T. Gutschner, et al., The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells, Canc. Res. 73 (3) (2013) 1180–1189.

[47] Q. Ji, et al., Long non-coding RNA MALAT1 promotes tumour growth and metastasis in colorectal cancer through binding to SF3B and releasing oncogenic PTB2 from SF3B/PTB2 complex, Br. J. Canc. 111 (4) (2014) 736–748.

[48] P.J. McCown, et al., Secondary structural model of human MALAT1 reveals multiple structure-function relationships, Int. J. Mol. Sci. 20 (22) (2019).

[49] M.C. Wang, et al., The regulatory structural model of MALAT1 becomes unstable in chronic myeloid leukemia and undergoes structural rearrangement in cervical cancer, Noncoding RNA 7 (1) (2021).

[50] L. Lipovich, et al., Activity-dependent human brain coding/noncoding gene networks, Genetics 192 (3) (2012) 1133–1148.

[51] K.M. Michalak, et al., Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth, Circ. Res. 114 (9) (2014) 1389–1397.

[52] M. Vausort, D.R. Wagner, Y. Devaux, Long noncoding RNA in patients with acute myocardial infarction, Circ. Res. 115 (7) (2014) 668–677.

[53] Z.H. Zhao, et al., Long non-coding RNA MALAT1 functions as a mediator in cardioprotective effects of fentanyl in myocardial ischemia-reperfusion injury, Cell Biol. Int. 41 (1) (2017) 62–70.

[54] J. Zhang, et al., Altered long non-coding RNA transcriptomic profiles in brain microvascular endothelium after cerebral ischemia, Exp. Neurol. 277 (2016) 162–170.

[55] J. Yao, et al., Long non-coding RNA MALAT1 regulates retinal neurodegeneration through CREB signaling, EMBO Mol. Med. 8 (4) (2016) 346–362.

[56] Q.S. Zhang, et al., Beta-asaore proteases protects MPP+-induced Parkinson’s disease via regulating long non-coding RNA MALAT1 and inhibiting alpha-synuclein protein expression, Biomed. Pharmacother. 83 (2016) 153–159.

[57] H. Ren, et al., Association of circulating long non-coding RNA MALAT1 in diagnosis, disease surveillance, and prognosis of acute ischemic stroke, Braz. J. Med. Biol. Res. 53 (12) (2020), e1974.

[58] F. Prinz, et al., The functions of the long non-coding RNA NEAT1 in non-cancerous diseases, Int. J. Mol. Sci. 20 (3) (2019).

[59] Z. Wang, et al., Long non-coding RNA NEAT1-centric gene regulation, Cell. Mol. Life Sci. 77 (19) (2020) 3769–3797.

[60] S. Armakolas, et al., MED16/lncRNA beta-coding RNAs are essential for structural integrity of nuclear paraspeckles, Proc. Natl. Acad. Sci. U. S. A. 106 (8) (2009) 2525–2530.
[93] R. Galupa, E. Heard, X-chromosome inactivation: a crossroads between chromosome architecture and gene regulation, Annu. Rev. Genet. 52 (2018) 295–326.
[94] N. Brockdorff, J.S. Bowness, G. Wei, Progress toward understanding chromosome silencing by Xist RNA, Genes Dev. 34 (11–12) (2020) 733–744.
[95] H. Fang, C.M. Diesteche, J.B. Berl贴心, X inactivation and escape: epigenetic and structural features, Front. Cell. Dev. Biol. 7 (2019) 219.
[96] N. Brockdorff, Localized accumulation of Xist RNA in X chromosome inactivation, Open Biol. 9 (12) (2019) 190213.
[97] E.B. Hook, D. Warburton, The distribution of chromosomal genotypes associated with Turner’s syndrome: birthweight prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism, Hum. Genet. 64 (1) (1983) 24–27.
[98] D. Smeets, et al., A cytogenetic and molecular study of a series of 45,X females and their parents, J. Med. Genet. 28 (3) (1991) 151–155.
[99] N. Brockdorff, et al., Conservation of position and exclusive expression of mouse Xist from the inactive X chromosome, Nature 351 (6324) (1991) 329–331.
[100] G. Borini, et al., Characterization of a murine gene expressed from the inactive X chromosome, Nature 351 (6324) (1991) 325–329.
[101] S.D. Brown, XIST and the mapping of the X chromosome inactivation centre, Bioessays 13 (11) (1991) 607–612.
[102] N. Brockdorff, et al., The product of the mouse xist gene is a 15 Kb inactive X-specific transcript containing No conserved Orf and located in the nucleus, Cell 71 (3) (1992) 515–526.
[103] C.J. Brown, et al., The human Xist gene expression: analysis of a 17 Kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus, Cell 71 (3) (1992) 527–542.
[104] A. Wutz, T.P. Rassmussen, R. Jaenisch, Chromosomal silencing and localization are mediated by different domains of Xist RNA, Nat. Genet. 30 (2) (2002) 167–174.
[105] Y. Hoki, et al., A proximal conserved repeat in the Xist gene is essential as a genomic element for X-inactivation in mouse, Development 136 (1) (2009) 139–146.
[106] D.P. Patil, et al., mi6A RNA methylation promotes Xist-Mediated transcriptional repression, Nature 537 (7620) (2016) 369–373.
[107] B. Moindrot, et al., A pooled shRNA screen identifies Rbm15, spen and Wtap as transcriptional repressor activities in X chromosome inactivation, Nature 469 (7368) (2010) 371–381.
[108] Y.L. Zhang, et al., The IncRNA XIST exhibits oncogenic properties via regulation of miR-449a and Bel-2 in human non-small cell lung cancer, Acta Pharmacol. Sin. 38 (3) (2017) 371–381.
[109] S. Diep, et al., Silencing of long noncoding RNA XIST attenuated Alzheimer’s disease-related BACE1 alteration through miR-124, Cell Biol. Int. 44 (2) (2020) 630–636.
[110] U. Braunschweig, et al., Dynamic integration of splicing within gene regulatory pathways, Cell 150 (2) (2012) 1292–1309.
[111] N. Brockdorff, et al., Development and validation of a murine X-inactivation model, Dev. Cell 39 (1) (2015) 246–259.
[112] C.A. McHugh, et al., The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3, Nature 521 (7551) (2015) 232–236.
[113] M.C. Wahl, C.L. Will, R. Luhrmann, The spliceosome: design principles of a dynamic RNP machine, Nat. Rev. Genet. 16 (4) (2009) 279–312.
[114] L.L. Chen, G. Shan, CircRNA in cancer: Fundamental mechanism and clinical potential, Canc. Lett. 505 (2021) 49–57.
[115] T.B. Hansen, et al., Natural RNA circles function as efficient microRNA sponges, Nature 514 (7521) (2014) 381–388.
[116] S. Mentink, et al., CircRNAs and circRNA exomes are a large class of animal genes with regulatory potency, Nature 495 (7441) (2013) 333–338.
[117] L.S. Kristensen, et al., The biogenesis, biology and characterization of circRNAs, Nat. Rev. Genet. 20 (11) (2019) 675–691.
[118] M. Zhou, et al., New progresses of circular RNA biology: from nuclear export to degradation, RNA Biol. (2020) 1–9.
[119] L.L. Patot, S. Wutt, S. Kadenber, Past, present, and future of circRNAs, EMBO J. 38 (16) (2019) e100836.
[120] Z. Song, G. Shan, CircRNA in cancer: Fundamental mechanism and clinical potential, Canc. Lett. 505 (2021) 49–57.
[121] J. Li, et al., Circular RNAs in cancer: biogenesis, function, and clinical significance, Trends Canc. 6 (4) (2020) 319–336.
[122] L.L. Chen, The expanding regulatory mechanisms and cellular functions of circular RNAs, Nat. Rev. Mol. Cell Biol. 21 (8) (2020) 475–490.
[123] S.J. Conn, et al., The RNA binding protein quaking regulates formation of circRNAs, Cell 160 (6) (2015) 1125–1134.
[124] R. Ashwal-Fluss, et al., Longish circular RNAs compete with pre-miRNA splicing, Mol. Cell 56 (1) (2014) 55–66.
[125] A. Ivanov, et al., Analysis of intron sequences reveals hallmark of circular RNA biogenesis in animals, Cell Rep. 10 (2) (2015) 170–177.
[126] M.A. Khan, et al., Digital RNA circular production from the titin gene, Circ. Res. 119 (9) (2016) 996–1003.
[127] L. Erriechelli, et al., FUS affects circular RNA expression in murine embryonic stem cell-derived neurons, Nat. Commun. 8 (2017) 14741.
[128] C.X. Liu, et al., Structure and degradation of circular RNAs regulate PKR activation in innate immunity, Cell 177 (4) (2019) 865–880 e21.
[129] J.W. Fischer, et al., Structure-mediated RNA decay by UPF1 and G3BP1, Mol. Cell 74 (2) (2020) 70–84.
[130] R. Jia, et al., Defining an evolutionarily conserved role of GW182 in RNA degradation, Cell Discov. 5 (2019) 450–457.
[131] O.H. Park, et al., Endoribonucleaseic cleavage of mi(6)as-containing RNAs by S.novae P/MRP complex, Mol. Cell 74 (2) (2019) 345–359.
[132] B.T. Hansen, et al., miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA, EMBO J. 30 (21) (2021) 4414–4422.
[165] C. Huang, et al., A length-dependent evolutionarily conserved pathway controls nuclear export of circular RNAs, Genes Dev. 32 (9-10) (2018) 639–644.
[166] Z. Li, M.G. Kourie, C. Huang, The nuclear export of circular RNAs is primarily defined by their length, RNA Biol. 16 (1) (2019) 1–4.
[167] Z. Li, et al., Exon-intron circular RNAs regulate transcription in the nucleus, Nat. Struct. Mol. Biol. 22 (3) (2015) 256–264.
[168] Y. Zhang, et al., Circular intronic long noncoding RNAs, Mol. Cell 51 (6) (2013) 792–806.
[169] L. Stoll, et al., A circular RNA generated from an intron of the insulin gene controls insulin secretion, Nat. Commun. 11 (1) (2020) 5611.
[170] N. Chen, et al., A novel FLI1 exonic circular RNA promotes metastasis in breast cancer by coordinately regulating TET1 and DNMT1, Genome Biol. 19 (1) (2018) 218.
[171] A. Bronisz, et al., The nuclear DICER-circular RNA complex drives the deregulation of the glioblastoma cell microRNAome, Sci. Adv. 6 (51) (2020).
[172] P. Xia, et al., A circular RNA protects dormant hematopoietic stem cells from DNA sensor cGAS-mediated exhaustion, Immunity 48 (4) (2018) 688–701 e7.
[173] W. Vermeulen, H. Lans, ISWI chromatin remodeling complexes in the DNA damage response, Cell 170 (2021) 469–469.
[174] Z. Li, et al., Exon-intron circular RNAs regulate transcription in the nucleus, Nat. Struct. Mol. Biol. 68 (4) (2017) 745–757 e5.
[175] M.D. Simon, et al., High-resolution Xist binding maps reveal two-step spreading during X-chromosome inactivation, Nature 504 (7480) (2013) 465–469.
[176] M.D. Simon, et al., The genomic binding sites of a noncoding RNA, Proc. Natl. Acad. Sci. U. S. A. 108 (51) (2011) 20497–20502.
[177] Y. Long, et al., How do lncRNAs regulate transcription? Sci. Adv. 3 (9) (2017), eaao2110.
[178] M. Hafner, et al., Transcriptome-wide identification of RNA-binding protein and microRNA target sites by PAR-CLIP, Cell 141 (1) (2010) 129–141.
[179] J.B. Lucks, et al., Multiplexed RNA structure characterization with selective 2'-hydroxyl acylation analyzed by primer extension sequencing (SHAPE-Seq), Proc. Natl. Acad. Sci. U. S. A. 108 (27) (2011) 11063–11068.
[180] Y. Ding, et al., In vivo genome-wide profiling of RNA secondary structure reveals novel regulatory features, Nature 505 (7485) (2014) 696–700.
[181] J.G. Dumelie, S.R. Jaffrey, Defining the location of promoter-associated R-loops at near-nucleotide resolution using bisDRIIP-seq, elife 6 (2017), e2644.
[182] P.A. Ginno, et al., R-loop formation is a distinctive characteristic of unmethylated human CpG island promoters, Mol. Cell 45 (6) (2012) 814–825.
[183] M. Sahz, et al., Prevalent, dynamic, and conserved R-loop structures associate with specific epigenomic signatures in mammals, Mol. Cell 63 (1) (2016) 167–178.
[184] M. Saiz, et al., R-ChIP using inactive RNase H reveals dynamic coupling of R-loops with transcriptional pausing at gene promoters, Mol. Cell 68 (4) (2017) 105–112.
[185] J. Konig, et al., icLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution, Nat. Struct. Mol. Biol. 17 (7) (2010) 909–915.
[186] X. Xu, et al., CircRNA inhibits DNA damage repair by interacting with host gene, Mol. Canc. 19 (1) (2020) 302–307.
[187] R.C. Spitale, et al., Transcriptome-wide RNA structure probing using FragSeq: transcriptome-wide RNA structure probing using high-throughput chemical probing of RNA secondary structure with next generation sequencing, Nucleic Acids Res. 42 (21) (2014).
[188] J.D. Loughrey, et al., SHAPE-Seq 2.0: systematic optimization and extension of high-throughput chemical probing of RNA secondary structure with next generation sequencing, Nucleic Acids Res. 42 (21) (2014).
[189] M.R. Mumbach, et al., HiChIRP reveals RNA-associated chromosome conformation, Nat. Methods 16 (6) (2019) 489–492.
[190] L.A. Sanz, et al., A circRNA from SEPALLATA3 regulates splicing of its cognate mRNA through R-loop formation, Nat. Plants 3 (2017) 17053.
[191] J.G. Underwood, et al., FragSeq: transcriptome-wide RNA structure probing using high-throughput sequencing (SHAPE-Seq), Proc. Natl. Acad. Sci. U. S. A. 108 (27) (2011) 11063–11068.
[192] R.C. Spitale, et al., Structural imprints in vivo decode RNA regulatory mechanisms, Nature 519 (7544) (2015) 486–490.
[193] J.D. Loughrey, et al., Fragmentation of RNA secondary structure probing using high-throughput sequencing, Nat. Methods 7 (12) (2010) 995–1001.