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Regulatory non-coding RNAs: revolutionizing the RNA world

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Abstract The majority of the genomic DNA sequence in mammalian and other higher organisms can be transcribed into abundant functional RNA transcripts, especially regulatory non-coding RNAs (ncRNAs) that are expressed in a developmentally and species-specific regulated manner. Here, we review various regulatory non-coding RNAs, including regulatory small non-coding RNAs (sncRNAs) and long non-coding RNAs (lncRNAs), and summarize two and eight kinds of distinct modes of action for sncRNAs and lncRNAs respectively, by which functional ncRNAs mediate the regulation of intracellular events.

Keywords Regulatory non-coding RNAs · RNA world · Modes of action

Introduction

Interestingly, the RNA world hypothesis suggests that in the early evolution of life, the source of life was RNA, which not only carries biological information, but can also carry out replication by self-catalysis [1]. With the constant evolution of life, the information-carrying function of RNA has been devolved to the more stable DNA; meanwhile, the RNA's catalytic functions have been replaced by versatile proteins. Thus, RNA is thought to primarily link genes and proteins, as indicated by the central dogma: 'DNA makes RNA makes protein'. However, several studies have shown that RNA continues to evolve and expand along with DNA and proteins. For example, with the development of various high-throughput and deep sequencing technologies, it is now known that there are only approximately 20,000 protein-coding genes in the enormous human genome; these genes constitute <2 % of the genome. Moreover, sequencing technology has revealed that the majority of DNA sequences of the mammalian genome can be transcribed into various functional RNA transcripts, especially various regulatory non-coding RNAs that play important roles in some cellular processes.

Based on length, regulatory non-coding RNAs are roughly classified into either small or long non-coding RNAs. Various ncRNAs exert their functions by special modes of action, mediating the regulation of various events. The modes of action of the various regulatory ncRNAs discussed in this review are listed in Table 1. Interestingly, some regulatory ncRNAs that are involved in long-distance trans-regulation are intercellularly and intracellularly mobile [2].

Here we systematically summarize modes of action of various regulatory ncRNAs and their roles in various cellular processes on the basis of studies conducted in recent years, providing a relatively comprehensive understanding of regulatory ncRNAs.

Regulatory small non-coding RNAs (sncRNAs)

Since the first small silencing RNA was discovered in 1993, large numbers of regulatory sncRNAs, which act as robust regulators of gene expression and genomic stability, have been sequentially identified. These ncRNAs can be roughly divided into 3 classes: microRNAs (miRNAs) [3],

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Table 1 Examples of Modes of action of regulative non-coding RNAs

ncRNA	Abbreviation	Length	Modes of action effects		References
Regulative small non-coding RNAs					
(1) microRNA	miRNA	~21 nt	PTGS (RNA degradation or translational arrest) CDGS (to a lesser extent)		[3, 7–12, 14–18]
(2) Endogenous small interfering RNA	Endo-siRNA	~21 nt	PTGS (RNA degradation or translational arrest) CDGS		[4, 7, 8, 13–18]
(3) Piwi-interacting RNA	piRNA	~29–30 nt	PTGS (RNA degradation) CDGS (to a lesser extent)		[5, 7, 8, 13–18]
Regulative long non-coding RNAs					
(1) SER3 regulatory gene 1	SRG1	~2.0 kb	Transcription activity itself	Inhibition	[33]
(2) PHO5 antisense ncRNA	–	~2.4 kb		Activation	[34, 35]
(3) Hox antisense intergenic RNA	HOTAIR	~2.2 kb	Mediate the changes of chromatin structure	Inhibition	[36, 39]
(4) KCNQ1 opposite strand/ antisense transcript 1	Kcnq1ot1	~9.2 kb			[43]
(5) HOXA transcript at the distal tip	HOTTIP	~3.7 kb		Activation	[36, 42]
(6) Growth arrest-specific 5	Gas5	~600 nt	Regulate assembly or activities of transcription factor-participatory complex	Inhibition	[44]
(7) Heat shock RNA 1	HSR1	~600 nt		Activation	[45]
(8) Erwinia virulence factor 2	Evf-2	~3.8 kb	Act as enhancer or isolator-like element	Enhancement	[47, 48]
(9) Steroid receptor RNA activator	SRA	~2.0 kb		Insulation	[49, 50]
(10) Nascent metastasis-associated lung aderocarcinoma transcript1	nascent MALAT1	8.7 kb (Homo sapiens)	Act as the precursors of small RNAs	Generate mascRNA	[51]
(11) X (inactive)-special transcript	Xist	~17 kb		Generate RITS/RISC	[52, 53]
(12) Xist antisense RNA	Tsix	~37 kb		-like complexs	
(13) H19 fetal liver mRNA	H19 lincRNA	~2.6 kb		Generate miR-675	[54]
(14) Zeb2 natural antisense transcript	Zeb2-NAT	>680 nt	Control the process of other RNAs	Mediates the alternative splicing	[55]
(15) Linc-muscle differentiation 1	linc-MD1	521 nt		Sponge function	[56]
(16) Half-STAU1-binding site RNAs	1/2-sbsRNA1-4	545–983 nt	Be involved in the degradation of mRNAs		[57]
(17) Terminal differentiation-induced ncRNA	TINCR	~3.7 kb	Mediate stabilization of differentiation mRNAs		[58]
(18) Noncoding RNA repressor of NFAT	NRON	2.7 kb (Homo sapiens)	Be involved in regulating the transport between cytoplasm and nucleus.		[59]

endogenous small interfering RNAs (endo-siRNAs) [4] and piwi-interacting RNAs (piRNAs) [5]. The most widely studied class of sncRNAs is ~22 nt miRNAs that, in animals, mediate post-transcriptional gene silencing. The size of endo-siRNAs is ~21 nt, which are specially processed from long dsRNAs without the short stem-loop structures. These endo-siRNAs negatively regulate the expression of targeting genes, whose transcripts are completely complementary to the sequence of an endo-siRNA. piRNAs are 24–30 nt ncRNAs in length, they are Dicer-independent and bind the PIWI subfamily of Argonaute family proteins, participate in maintaining genome stability in germline cells [6]. So far, >1,872 miRNAs, >199 endo-siRNAs, and >1,005 piRNAs are discovered and extensively studied based on miRBase, piRNABank. These three classes of sncRNAs differ in their biosynthesis approach, mode of regulation and sncRNA-mediated biological pathways [7]. However, the interconnectedness of distinct sncRNAs pathways is now accepted, and there are instances of competition or cooperation among these sncRNA pathways during the regulation of gene expression and protection of the genome from various endogenous and exogenous threats [8]. sncRNAs are approximately 20–30 nt in length and are closely linked with the

Argonaute (Ago) protein family members. They guide Ago to special target sites, which then typically lead to the down-regulation of target gene expression.

Regulatory sncRNAs mainly function through the two following modes: (1) the most important and common mode is that regulatory sncRNAs are loaded into the RISC (RNA-induced silencing complex), mediating homologous mRNAs degradation or translational arrest. This is post-transcriptional gene silencing, in which miRNAs may primarily mediate translational arrest by incomplete pairing between miRNAs and target mRNAs to block different phases of mRNA-mediated translation (especially at the translation initiation phase). miRNAs are thought to mediate the arrest of transcription initiation by inhibiting the function of eukaryotic initiation factor 4E/Cap and poly (A) tail [9]. Meanwhile, there are also reports that miRNAs influence or inhibit the translational elongation velocity [10, 11]. However, mammalian miRNAs may predominantly act to decrease target mRNA levels [12]. Compared with miRNAs, siRNAs and piRNAs usually approximately perfect match with homologous mRNAs, mediating the degradation of target mRNAs through the TRAMP polyadenylation pathway [13]. This process allows an exosome, consisting of a 3′–5′ exonuclease complex and related proteins, to mediate the mRNA degradation process, such as by shortening the poly (A) tail or by de-capping (Fig. 1a). Interestingly, sncRNA-mediated translational arrest is closely linked with mRNA degradation; through a mutual-coordination relationship: miRNA-mediated translation arrest is often followed by mRNA deadenylation and decay [14]. For example, translational arrest occurs prior to miR-430-mediated mRNA degradation during Zebra fish development [15]. (2) On the other hand, various regulatory sncRNAs can also mediate chromatin-dependent gene silencing (CDGS). CDGS includes transcriptional gene silencing (TGS) and co-transcriptional gene silencing (CTGS), or cis-PTGS, in which snc-RNAs are loaded into the RITS (RNA-induced transcriptional silencing complex). For example, the inactivation of some promoters in the centromeric DNA repeats is achieved mainly through CTGS, in which non-coding centromeric transcripts (CenRNA) are cut and then loaded into the RITS. Meanwhile, the RNA-directed RNA polymerase complex (RDRC) amplifies the silencing effect. RDRC are in competition with the TRAMP polyadenylation pathway that targets aberrant RNAs for degradation by a 3′ → 5′exonuclease complex, which is called the exosome (Fig. 1b). In addition, the Clr4 H3K9 methyltransferase complex (CLRC) participates in the formation of inhibitory H3K9me3, mediating the formation of heterochromatin, which is achieved by regulatory sncRNA-mediated TGS [16, 17]. Regulatory sncRNAs can also directly base-pair with cognate unwound DNA regions, thus mediating transcriptional silencing. Furthermore, the nascent ncRNAs transcribed from an antisense sequence or upstream sequence of their target gene promoters use the TGS strategy to mediate silencing (Fig. 1c). For example, miR-320 is the product of the antisense transcription of the cell cycle gene POLR3D promoter. It directly interacts with RNAi protein-AGO1 and PCG complex component- EZH2 (EZH2 catalyzes the formation of H3K27me3) and targets them to the adjacent POLR3D promoter to mediate the formation of inhibitory epigenetic markers [18]. Regulatory sncRNA-mediated TGS participates in heterochromatin formation and specific gene silencing through the recruitment of various histone modification enzymes or DNA methylase to the target sites, changing the epigenetic environment of the target sites to achieve silencing regulation.

Long non-coding RNAs (lncRNAs)

lncRNAs are arbitrarily identified as the >200 nt ncRNA molecules with little or no protein-coding potential. They occupy a considerable portion of whole ncRNAs and are widespread in the nucleus and cytoplasm. Most lncRNAs are transcribed from loci with a chromatin signature (such as H3K4me3 or H3K36me3), indicating that their transcription is subject to cell-type-specific dynamic regulation [19]. Biophysical analysis shows that many lncRNAs can fold into many functional secondary structures through which they may exert their biological functions [20]. For many years, these lncRNAs were thought to be the by-product of transcriptional noise; however, a large number of studies have shown that various lncRNAs are subject to regulation during development [21, 22], especially early embryonic development [23, 24]. Moreover, lncRNAs also show cell-type-specific expression patterns and are targeted to specific subcellular regions. In recent years, a large number of functional lncRNAs have been sequentially discovered [25]; they are involved in the dynamic regulation of higher chromosome structure, telomere biology, organization of subcellular structures and regulation of gene expression, especially the regulation of adjacent protein-coding gene expression. These lncRNAs are often associated with human diseases [26–30]. There characteristics suggest that lncRNAs are not “noise” but functional molecules, playing important roles in various cellular processes. Importantly, not all lncRNAs are regulatory lncRNAs, and some lncRNAs are constitutively expressed. For example, MEN epsilon/beta [31] and Xlsirts [32] are involved in the establishment of nuclear paraspeckles and organization of the cytoskeleton, respectively. So far, >127 lncRNAs are discovered and extensively studied based on LncRNA Database.

Compared with regulatory sncRNAs, regulatory lncRNAs display a much wider variety of modes of action: (1)

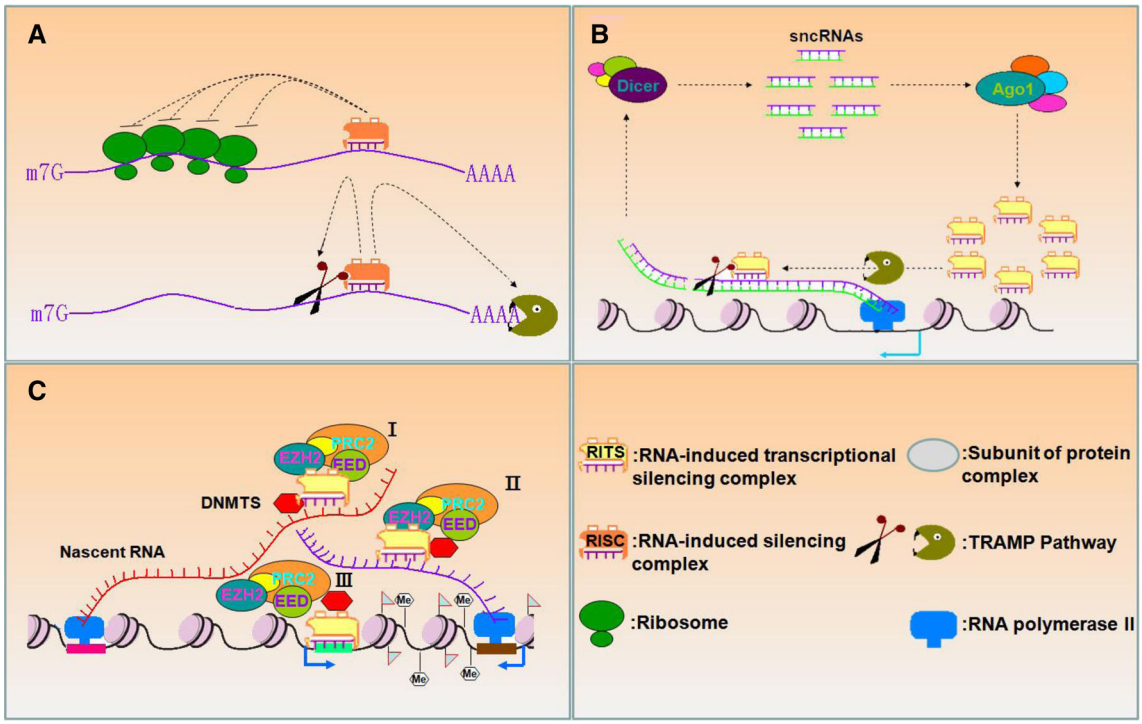

Fig. 1 Action modes of the regulatory sncRNAs. **a** Regulatory sncRNAs are loaded into RISC (RNA-induced silencing complex), mediating matched mRNAs' translational inhibition or degradation. **b** Regulatory sncRNAs are loaded into RITS (RNA-induced transcriptional silencing complex), which mediates the co-transcriptional gene silencing (CTGS) of special locus, achieving gene silencing. **c** Regulatory sncRNAs are loaded into RITS that mediates the transcriptional gene silencing (TGS) of special locus, achieving transcription inhibition

the transcriptional activity of regulatory lncRNAs affects the transcriptional activity of the downstream protein-coding gene. For example, the transcription of lncRNA-SRG1 (SER3 regulatory gene 1) passing through the downstream protein-coding gene SER3′s promoter region will directly interfere with the transcription factors' ability to bind the promoter in the Saccharomyces cerevisiae, thereby preventing SER3 gene expression [33]. On the other hand, the lncRNA's transcription itself will also relax the tightly packed chromatin conformation of the downstream protein-coding genes, promoting transcription. For example, in yeast, a 2.4 kb antisense ncRNA transcribed from the PHO5 3′ end will affect the exchange/turnover of nucleosomes in the vicinity, which allows the nucleosomes to eject. This results in loosening of the chromatin conformation of the adjacent protein-coding gene PHO5, improving the accessibility of transcription factors and thus promoting transcription [34, 35] (Fig. 2a). (2) Regulatory lincRNA indirectly controls transcription by mediating changes in the chromatin structure, especially the epigenetic environment [36], where the classic example of lincRNA-mediated transcriptional silencing is the ~2.2 Kb HOTAIR (Hox antisense intergenic RNA). HOTAIR is transcribed from the HOXC locus of Chromosome 12 and acts as an RNA scaffold that can simultaneously combine with multiple histone modification enzymes. HOTAIR targets Polycomb repressive complex 2 (PRC2) [37] and Lysine Specific Demethylase1 (LSD1) [38] to the HOXD locus to mediate the formation of inhibitory H3K27me3 and the removal of active H3K4me3, resulting in transcription silencing [39]. In contrast, lncRNAs can also promote gene expression through a similar mode. For example, the ~3.7 kb HOTTIP (HOXA transcript at the distal tip) transcribed from the terminal of the mammalian HOXA locus can recruit the WDR5-MLL (mixed lineage leukemia) [40, 41] complex to the target site to promote the formation of H3K4me3, thus activating transcription [42]. Chromosome conformation capture carbon copy assays show that regardless of whether HOTAIR or HOTTIP mediates long-distance intrachromosomal or interchromosomal regulation, they both achieve regulation by the formation of long-range looping interactions (Fig. 2b). It was recently reported that long ncRNAs also can mediate the establishment of site-special DNA methylation, for example Kcnq1ot1 ncRNA participate in guiding and maintaining the CpG methylation at somatic differentially methylated regions flanking the UIGs (ubiquitously imprinted genes) [43]. (3) Regulatory lincRNA directly regulates the assembly or activities of the transcription factor-participatory complex. For example, the ~600 nt

Gas5 (growth arrest-specific 5) transcribed by POL II specifically aggregates in growth-arrest cells, and it possesses pro-apoptotic effects through the inhibition of glucocorticoid-mediated anti-apoptotic gene expression. Gas5 can mimic a GR (glucocorticoid receptor)-binding site, which can specifically combine with GR and prevent it from binding to the GRE element at anti-apoptotic gene promoters to inhibit transcription [44]. On the other hand, under heat-shock conditions, the ~600 nt ncRNA HSR1 (heat shock RNA 1), which lacks a poly (A) tail, participates in trimerization of HSF1 (heat shock transcription factor 1) and enables the acquisition of a site-specific DNA-binding activity to achieve transcriptional activation [45] (Fig. 2c). In addition, regulatory ncRNAs are also involved in the recruitment of basic transcription machinery to specific promoters. For example, the interaction between the lncRNA transcribed from the upstream locus of the dihydrofllate reductase (DHFR) gene and DHFR's main promoter forms a triple complex, which inhibits the binding of co-transcriptional molecule TFIID and prevents the formation of a pre-initiation complex [46]. (4) Regulatory lincRNAs can acts as enhancer or isolator-like elements. For example, the lncRNA-Evf2 (Erwinia virulence factor 2) transcribed from an ultra-conserved long-range enhancer can recruit transcription factor DLX2 to this enhancer and simultaneously induce the transcription of the adjacent protein-encoding gene DXL6 [47, 48]. Similarly, lncRNA-SRA (steroid receptor RNA activator) interacts with RNA helicase p68 and CTCF (CCCTC binding factor, a DNA-binding protein required for genome-wide transcriptional isolation), targeting them to specific DNA sequences. Thus, SRA plays an important role in the isolation function of insulators [49, 50] (Fig. 2d). (5) Regulatory lincRNAs can act as the precursors of many small RNAs. For example, a 7 kb nascent ncRNA-MALAT1 (metastasis-associated lung aderocarcinoma transcript1) is excised into two RNA molecules by RNase P. One of these molecules is a mature-type long MALAT1, which is localized to nuclear speckles. The other is a highly conserved 61 nt tRNA-like sRNA (mascRNA), which is eventually transported to the cytoplasm [51]. Furthermore, recent reports show that many development-regulatory sRNA (25–42 nt) can be mapped into Xist and Tsix loci, which is reminiscent of the following hypothesis: During X chromosome selective inactivation in female mammalian cells, the hybridization of Xist and Tsix forms a long double-strand RNA, which is cut into 25–42 nt dsRNAs. These dsRNAs are further processed into RITS/RISC-like complexes, mediating X chromosome inactivation through a similar sncRNA-mediated silencing mechanism [52, 53] (Fig. 2e). Interestingly, regulatory sncRNAs also can derive from some special lncRNAs, for example the H19 lincRNA is a developmental reservoir of miR-675, which is embedded in H19′s first exon [54]. (6) Regulatory lincRNAs control the processes of other RNAs. For example, during epithelial-mesenchymal transition (EMT), the Zeb2 natural antisense transcript (Zeb2-NAT) mediates the alternative splicing of E-cadherin's transcriptional inhibitor Zeb/sip1 mRNA. When EMT occurs, NAT expression is induced. NAT then binds to the 5′end of an intron of the Zeb/sip1 mRNA (This intron contains an internal ribosome entry site-IREs that is required for Zeb/sip1 translation). NAT binding prevents the spliceosome from removing this IREs-containing intron from the mature mRNA, which eventually allows the expression of Zeb/sip1 [55]. In addition, some lncRNAs utilize a "sponge function" to regulate various sRNAs. For example, during muscle differentiation, a specific lncRNA (linc-MD1) can "sponge" miR-133 and miR-135, which regulate the expression of two key transcription factors,- MAML1 and MEF2C [56] (Fig. 2f). (7) Regulatory lincRNAs are also involved in the degradation of mRNAs. For example, a specific lncRNA (1/2-sbsRNAs, half-STAU1-binding site RNAs) can mediate SMD (staufen1 (STAU1)-mediated mRNA decay), where the STAU1-binding site is formed by the interaction between this lncRNA's AluU element and the target mRNA Alu element [57] (Fig. 2g). Interestingly, the similar mode of action is utilized to mediate stabilization of differentiation mRNAs by special lncRNAs. For example 3.7 kb lncRNA TINCR (terminal differentiation-induced ncRNA) that controls human epidermal differentiation [58]. (8) Regulatory lincRNAs are involved in regulating the transport between the cytoplasm and nucleus. For example, calcium-regulatory transcription factors NFATs (nuclear factor of activated T cells) proteins are highly phosphorylated in resting cells and are located in cytoplasm. Their cytoplasmic localization is regulated by a large cytoplasmic RNA–protein scaffold complex, which mainly consists of a special lncRNA (NRON, noncoding RNA repressor of NFAT). When the concentration of Ga2+ in cytoplasm is increased, Calcineurin (Ga2+/calmodulin-dependent phosphatase) catalyzes the dephosphorylation of NFATs, resulting in separation of NFATs from the RNA-Scaffold. Then, the NFATs are transported into nucleus, promoting the expression of related genes [59] (Fig. 2h).

Roles of noncoding-RNAs in various cellular processes

Regulatory ncRNAs are involved in various cellular processes, most of which are known to be involved in different phases of gene expression regulation (including before RNA transcription and at the transcriptional and post-transcriptional level) that affect the expression of protein-coding genes. In addition, regulatory sncRNAs are involved in the

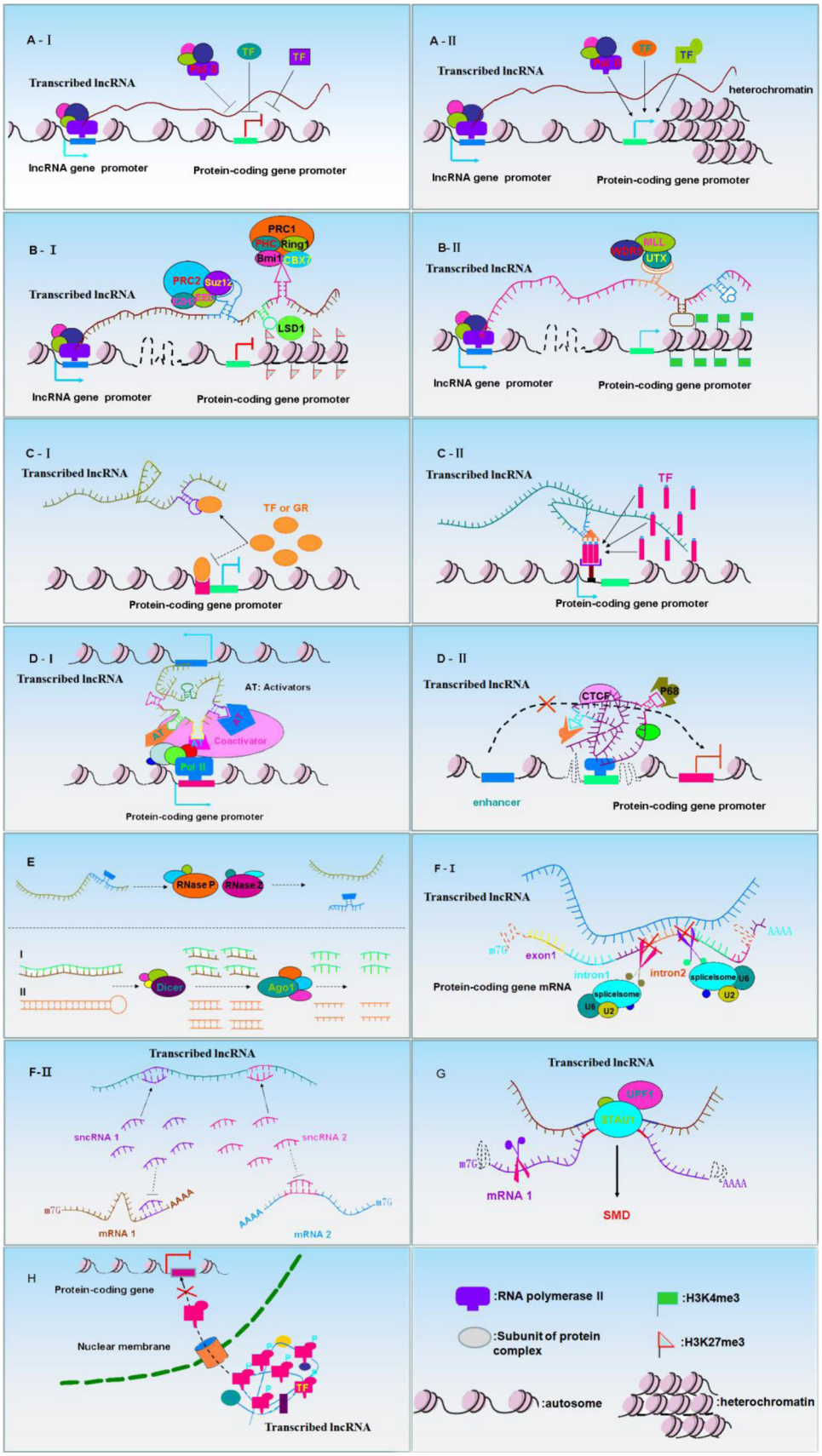

Fig. 2 Action modes of regulatory lncRNAs. **a**-I, II The transcriptional activity of regulatory lncRNAs affects the transcriptional activity of the downstream protein-coding gene, achieving transcriptional inhibition and activation by interfering with the transcription factors' accessibility and relaxing the tightly packed chromatin conformation respectively. **b**-I, II Regulatory lincRNA indirectly controls transcription by mediating changes in the chromatin structure especially the epigenetic environment, achieving transcription silencing or activating by targeting multiple histone modification enzymes to special locus. **c**-I, II Regulatory lincRNA directly regulates the assembly or activities of the transcription factor (TF)-participatory complex, promoting transcriptional inhibition and gene expression by competitively combining with TFs and assisting the assembly of TFs respectively. **d**-I, II Regulatory lincRNAs can acts as enhancer or isolator-like elements, promoting gene expression or silencing respectively. **e** Regulatory lincRNAs can act as the precursors of many small RNAs. **f**-I, II Regulatory lincRNAs control the processes of other RNAs (e.g. mRNA's alternative splicing and miRNA-mediated silencing regulation). **g** Regulatory lincRNA mediates SMD by the STAU1-binding site formation between lncRNA's AluU element and the target mRNA's Alu element. **h** Regulatory lincRNAs are involved in regulating the transport between the cytoplasm and nucleus

maintenance of genome integrity. They not only participate in the establishment of various types heterochromatin, preventing some parasitic DNA sequences from reactivating, but they also mediate the inactivation of exogenous pathogens, especially various mobile sRNAs that mediate long-distance silencing effect. Recently, some miRNAs were reported to mediate the effects of morphogen gradient during early development [60, 61]. Similarly, various piRNAs are involved in the normal development of germ cells and ensuring germ line stability by inhibiting transposons. LncRNAs also possess some special functions, such as the sponge function, by which some lncRNAs regulate various sncRNA-mediated silencing pathways. Furthermore, lncRNAs often act as special ceRNAs (competing endogenous RNA) [62, 63], which plays an important role during development and tumorigenesis.

Conclusion

Technological improvements promote the discovery of various new classes of regulatory non-coding RNAs, which are constantly revealing a mysterious RNA world. Many studies demonstrate not only that RNA is an intermediate between genes and proteins but also that it evolved alongside DNA and proteins. On the one hand, biological genetic information is amplified by RNA editing, mRNA recoding, and high RNA mutation rates. On the other hand, organisms also evolve with various function-specific RNAs, such as transfer RNA (tRNA) that can target amino acids to a specific mRNA site, ribosomal RNA (rRNA) that can catalyze the formation of a peptide bond, messenger RNA that act as the template for protein translation, and regulatory ncRNAs that are primarily involved in the complex regulation of gene expression. These functional ncRNAs exert their functions by various special modes of action, especially various regulatory ncRNAs.

The types and quantity of regulatory ncRNAs are constantly expanding as the developmental and physiological complexity of species increases. However, the evolution of protein-coding genes is much slower; thus, regulatory ncRNAs play an important role in the creation of greater developmental and physiological complexity. Regulatory ncRNAs directly or indirectly mediate the regulation of various cellular processes, especially different stages of gene expression, through special modes of action. The high mutation rate of RNA promotes the rapid evolution of regulatory ncRNAs, which is beneficial not only in allowing organisms to adapt to changes in the environment but also in rapidly developing various special modes of action and new regulatory ncRNAs. Perhaps this is why most of the regulatory ncRNAs have limited sequence conservation. However, the low expression and limited sequence conservation of regulatory ncRNAs, especially lncRNAs, increases the difficulty of studying of functional ncRNAs. Therefore, regulatory non-coding RNAs of functional-diversity and abundance revolutionize the RNA world.

With the development of various high-throughput and high-resolution sequencing technologies, a large number of functional ncRNAs that play an important role during development and pathogenesis have been identified. Perhaps some diseases with unknown etiology are the result of regulatory ncRNA abnormalities [64]. In future studies, various special regulatory ncRNAs may become targets for drug development.

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