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# Paradigm shift in molecular biology; THE EMERGANCE OF REGULATORY RNA, Review:

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**ABSTRACT:**
The discoveries over the last ten years demonstrate an evolution in cell and molecular biology. Non-protein coding RNAs, also known as the "hidden layer of gene regulation," are receiving more and more attention as regulators of gene expression. But outside of model organisms, very little is known regarding the existence and function of such regulatory RNA genes. Ribonucleic acid has a variety of tasks in the control and organization of gene expression in higher organisms, in addition to acting as an intermediary or encoder between DNA and proteins and only encoding proteins, according to studies and other evidence. Regulatory Ribonucleic acidS perform numerous crucial tasks at practically every level, but they are particularly crucial in the epigenetic processes that influence differentiation and development. The advent in the formation of regulatory RNA also points to the critical function of RNA in human development, differentiation, and evolution.

**Keywords:** Regulatory RNA, siRNA, Molecular Biology, Paradigm shift, gene regulation.

## I. INTRODUCTION:

Regulatory RNAs are the small class of RNAs that has the role in gene regulation. This class comprises the types of RNAs which are non-coding and small, but are highly structured. Following various mechanisms these RNAs play a vital role in gene regulation. The mechanisms include protein target binding, the modification of proteins, binding to mRNA targets. From long RNA has been the basic center of molecular biology and was considered as the earliest life molecule performing the dual role as catalytic and informational functions. It is assumed that its informational functions were converted into more stable form of DNA and its catalytic functions are converted into polypeptides. With the entry of chemists into biology in the 1940s, the idea was generated that the role of RNA is intermediary between DNA and proteins. The idea that genes encoded the enzymes which are the functional components of cells itself had deeper roots in the era before the extensive knowledge of the use of digital information for systems control. Even after the rejection of one gene one protein theory after the discovery of alternate splicing in 1970s, the protein centric view in molecular biology remains same. It was supported by the assumption that changing's or mutations affects the cis-acting binding sites of proteins. However, the rise of RNA interference and nuclear introns challenged that view. The discovery of high screening methods helps in the identification of different types and large numbers of RNAs. Recent evidences suggest that in humans the number of genes encoding regulatory RNAs is more than the number of genes encoding proteins [1, 2].

**The role of RNA, early ideas:**

After the clear knowledge of DNA helical structure in 1953, the following years were known as the era of decoding genetic code and discovering the pathway between gene and polypeptide. Crick published in 1953, the central dogma which describes the flow of genetic information that is DNA makes RNA makes protein. This central dogma proved to be accurate and long lasting and includes the assumption of reverse transcriptase. In the mid of 1950, the relationship between ribosomal RNA and ribosomes known as the platform for protein synthesis was established. In 1958, the role of tRNA and in the 1961, the role of mRNA was experimentally confirmed. Various studies in the following years confirmed that proteins are not only the enzymes but also the center of cellular machinery [3-7]

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**The advent of small nuclear RNAs:**

Other small classes of small nuclear DNA were identified just after the advent of transfer RNAs and ribosomal RNAs and their functional elucidation. Among them, many sRNAs were seen to be a part of RNPs (ribonucleo-protein complexes). It was later found that one class of small nuclear RNA was the basic co factor in splicing of RNA, hence they got the designation of spliceosome RNAs. In the association of RNA-RNA and RNA-proteins, various snRNAs such as U1, U2, U3 etc. plays a vital role.

Other types of small RNAs were seen to be localized to nucleoli and also had the function for guiding methylation as well as pseudo-uridylation of ribosomal RNA, transfer RNA (fig 1). In pre RNA splicing anf maturation of mRNA and tRNA, the modification of tRNA, rRNA and snRNAs played essential roles. [8-13]

**The evolution of diverse nuclear RNAs and introns:**

The first clue of the additional role of RNA in higher organisms was the identification of "heterogeneous" nuclear RNA. The presence of hnRNA and the associated invention of the huge number of repeating sequences (different classes of retro-transposed sequences with similar makeup which engage large proportions of plant and animal genomes) led Britten and Davidson to give assumption in the 1969 those cells of animals might have extensive RNA-dependent regulatory channels. While at that time this assumption captivated a huge deal of interest, it also quickly terminate, with the promoters not re-visiting it even after the successive invent of non-coding transcripts called introns, instead focusing on regulatory channels managed by transcription factors or the importance of transposons in evolution of proteins.

The great advent in the history of the cell and molecular biology was the invention of introns in 1977. Because before introns no one cannot imagine that genes of higher organisms would be interrupted by introns. It was assumed that non-coding ribonucleic acids were broken down although at that time it was not confirmed. Whatever the case is, the introns were broken down and become the debris known as genomic debris and their existence has justified as evolutionary remains which have role in pre-biotic regulation gathering of polypeptide-coding RNAs that have lingered in higher organisms. [14-17]

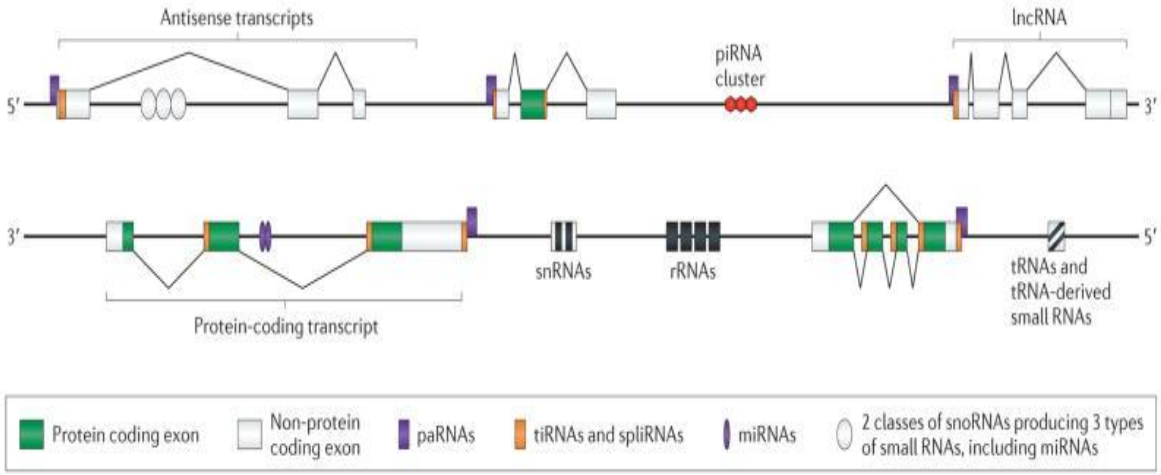


Figure 1: Expression of regulatory RNA: Diagrammatic representation of the transcriptional landscape of mammals with genes expressing coding and non coding transcripts.

**Catalytic activity of RNA:**

A few years later after the discovery of introns, it was found that RNA itself has capacity of enzymatic catalysis. This proved the evidence that catalysis of ribonucleic acids also goes on which is remained in particular circumstances particularly at the key of RNA splicing and translation of mRNA. This strengthen the concept of cell biology that the role of RNA is to act as a tribune of production of proteins. Besides all these evidences there was not any clue that RNA functions as a regulatory factor. Infact there is

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evidences that catalytic RNA is found in animals, introns, plants and UTRs and these RNAs play various roles such as regulation of reactions of post transcriptional cleavage. [18-20]

**The revolution of snRNAs:**

**MicroRNAs discovery:**

Ambros and colleagues declared the first evidence of small regulatory RNAs was showed by in the 1993. The evidence was collected after the discovery of genetic loci that regulates the timing of Caenorhabditis elegans development that is lin-5 and let-7. Very few micro RNAs have been discovered from let-7 because it is highly conserved from nematodes to humans. All such RNAs remained engrossing factors until the findings of interference RNAs. Now-a-days there are a huge number of micro RNAs in databases. Among them almost all had eluded prior detection by genetic screens. On the other hand, many micro RNAs can be detected by conservation; it is further evident that many are lineage and tissue specific. There are also many evidences that micro RNAs can regulate large numbers of target mRNAs and inversely many mRNAs have target sites for micro RNAs. mRNAs are mostly targets of micro RNAs but, other RNAs may also be the target of miRNAs. It has been shown that miRNAs are involved in the regulation of many developmental and disease process such as pluripotency, diabetes etc. [21-30]

**The pathway of RNA interference:**

miRNAs are just one facet of the RNA interference phenomenon, that causes splicing of expression of genes just after introducing sense and antisense RNA pairs. All such discoveries were augured because of the critical process of transgene silencing, particularly in plants. This was correlated with antisense RNA and sRNA directed deoxyribonucleic acid methylation of transgenes. The analysis of mechanism behind this showed that exogenous dsRNA was converted into small fragments of same size to microRNAs. This analysis suggests that micro RNAs may have a similar inducible system and leads towards the rise of precursors present in the stem loop structure. This also led to the discovery of key genes as well as enzymes that are involved in their biogenesis and functions particularly Drosha and dicer proteins. Drosha proteins have the role in cutting and transport of double stranded RNA from the nucleus to cytoplasm. In cytoplasm they are further modify by dicer and converts into small dsRNA. After modification among two strands sRNAs only one is packed into component called Ago-component, which is the part of RNA induced splicing complex (RISC). The small RNA strand that is complementary to RNA targets, guide the RISC. The current view suggests that small interference RNAs functions by exact pairing and by Ago splitting of target RNA. These small interference RNA occurs more frequently in plants. As compared to there is an incomplete homology with respect to their targets in case of micro RNAs. Micro RNAs acts primarily at the levels of translation.

There is an assumption that Micro RNAs as well as small interference RNAs acts post-transcriptionally and in cytoplasm. Despite this assumption, the presence of Ago components in the nucleus and also the role of the pathway of RNAi in regulation of epigenetics suggest that there is more complex system than expectations. In addition to this there is also evidences of other interesting pathways, for example; editing and modification of RNA and the pathways [31-37].

**Small RNAs (PIWI associated):**

In addition to Ago proteins associated with small and micro RNAs, there is also a subclass of Argonaute proteins also called as Piwi proteins. These proteins are essential in germ cell development. These proteins are associated with slightly different class of small RNAs of about 26-30 nt. This subclass act to silence transposons in germ cells either by epigenetic ways or post transcriptionally. Piwi associated RNAs are particularly found in the nucleus where they co-localize in a manner that is RNA dependent in association with poly-comb protein groups. They also appear to express in other tissues as well such as the brain. Their presence in other tissues suggests that they have the role beyond protection of the genome in epigenetic processes.

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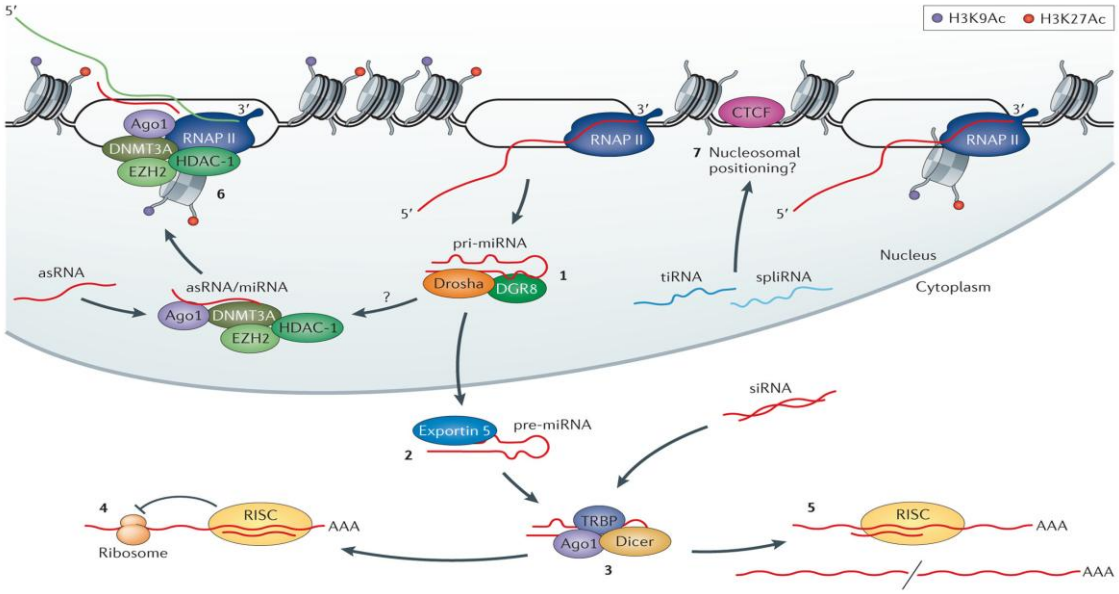


Figure 2 small regulatory RNA functional pathways:

**Other eukaryotic small RNAs**

While RNAi systems are still being unraveled in biochemistry, molecular genetics and biology, but indicate widespread but ancient systems that controls many cellular processes, whose investigations are still under process. These adopted systems includes variations which are lineage specific for example, "U21" RNA which are present in C. elegans. To great surprise it appears that 3 different classes of small RNAs are produced in fission from yeast to human by all snoRNAs. From these three classes one is of almost same length and roles as micro RNAs. The other class is similar to piwi RNAs. Besides this there are also interesting and captivating reports that fragments of transfer RNA build in tissue specific manner and they have associations with Ago proteins. In recent studies another class of small RNAs has been revealed from sequencing of small RNAs not in plants but only in animal cells. This discovered class contains 17-18 nt RNAs in length that are associated with transcription initiation. Still there is no knowledge about the origin and role of these RNAs, but studies and evidences revealed that they might be involved in positioning of nucleosomes and in different stages of organization of chromatin. Another class of promoter associated RNAs are also found in certain studies which are called as PARs and they may perform functions in ribonucleic acid directed silencing of genome.

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Table 1 Years of discovery

| | |
|---|---|
| 1941 | One gene-one enzyme. |
| 1953 | Double helical structure of DNA describe |
| 1958 | Central dogma proposed by Francis Crick |
| 1961 | mRNA confirmed as intermediate between protein and DNA |
| 1961 | Jacob and Monod speculate that the *lac* repressor is an RNA |
| 1966 | Discovery of heterogenous nuclear RNA |
| 1969 | Model proposed for RNA acting in intermediate fashion in gene regulation |
| 1972 | hnRNAs, chromosomal RNAs shown to be functional without making protein |
| 1977 | Intron ncRNA elements defined |
| 1982–3 | Self Splicing catalytic RNAs |
| 1989 | Transgene silencing observed in plants |

| | |
|---|---|
| 1990 | Transgene silencing linked to antisense RNA |
| 1990 | H19 ncRNA discovered |
| 1992 | Xist ncRNA discovered |
| 1993 | Lin-4 miRNA discovered |
| 1994 | Regulatory RNAs proposed to be central to animal evolution and development |
| 1994 | RNA directed DNA methylation observed in plants |
| 1998 | RNA interference described in plants and animals |
| 1999 | Tsix, antisense transcript to Xist described |
| 1999 | Small RNA required for PTGS in plants |
| 2000 | Let-7 miRNA discovered |
| 2001 | Dicer described involved in RNAi |

Table 2 Year of discoveries

| | |
|---|---|
| 2001 | RNAi (PTGS) found functional in human cells |
| 2001 | Regulatory RNA networks proposed to control epigenetic processes |
| 2002 | First reports of large numbers of noncoding RNAs in animals |
| 2002 | AIR antisense RNA involved in imprinting |
| 2003 | Drosha described in miRNA processing |
| 2004 | Small RNA shown to epigenetically control transcription (TGS) in human cells |
| 2004 | Argonuate 2 directs catalysis in RNAi in mammals |
| 2005 | piRNAs described |
| 2005 | Confirmation of large numbers of long noncoding RNAs in mammals |
| 2005 | ~70% of sense transcripts have antisense counterparts, some show function |
| 2005 | Discovery of the CRISPR system of bacterial RNA-based defence |

| | |
|---|---|
| 2006 | Antisense RNA TGS shown to require DNMT3a, EZH2, HDAC- |
| 2006 | Argonautes 1 and 2 found involved in RNA-directed TGS in human cells |
| 2006 | ncRNAs involved in trithorax regulation. |
| 2007 | HOTAIR shown to play a role in development and associate with polycomb |
| 2008 | Long antisense RNAs found to epigenetically regulate sense counterparts |
| 2008 | LncRNAs shown to interact with trithorax and activated chromatin |
| 2008 | Hundreds of lncRNAs shown to have specific expression in brain |
| 2009 | tiRNAs reported at transcription start sites in mammals |

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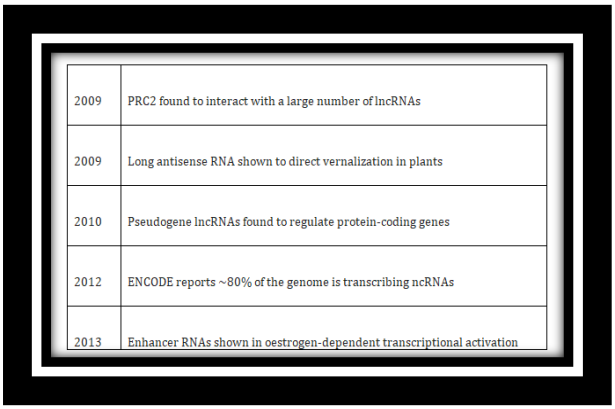

| | |
|---|---|
| 2009 | PRC2 found to interact with a large number of lncRNAs |
| 2009 | Long antisense RNA shown to direct vernalization in plants |
| 2010 | Pseudogene lncRNAs found to regulate protein-coding genes |
| 2012 | ENCODE reports ~80% of the genome is transcribing ncRNAs |
| 2013 | Enhancer RNAs shown in oestrogen-dependent transcriptional activation |

**Prokaryotic Non-coding RNAs:**

In bacterial species, a large number of small RNAs have been discovered and have a role in regulation of the vast variety of adaptive responses. The small regulatory ribonucleic acids of bacteria follow a simple pathway of antisense mechanisms. These RNAs functions by regulating translations and target mRNAs stability by alternation in their secondary structures. Cis acting regulatory sequences are also identified in bacteria in various different studies and evidences. All these sequences act allosterically by metabolite binding in order to regulate gene expressions. The kingdom of prokaryotes again surprised us recently, in terms of delicate molecular machinery. Many bacteria as well as an archaeal genome contain other DNA sequences in addition to their own which they derived from viruses. These sequences are now termed as Clustered regularly interspersed short palindromic repeats more commonly known as CRISPR. CRISPR sequences by incorporation of viral DNA in between repeats acts as innate immune system. This incorporated viral DNA later transcribed and produce guide RNAs. This system is now widely used by many biotechnologists in genome editing processes for achieving various targets one is to cure genetic disorders.

**Long regulatory RNAs:**

**Eukaryotic long regulatory RNAs:**

In 1994, Mattick proposed that there are some genes that are evolved for expressing only noncoding RNAs. He suggest that these ribonucleic acid-based regulatory systems was basic requirement for the appearance of higher organisms. With the help of high throughput array and sequencing techniques it was revealed that many loci in mammals in addition to coding regions also contains large non coding interrupts that does not code for any protein. In 2005 the initial findings for the presence of such large regions were confirmed and these findings were extended by ENCODE project. All these findings showed that majority of human and mouse genome was transcribed in one and other way. Similar findings was obtained in studies with other different organisms. It was assumed that the percentage of transcriptome increases with complexity of development because most intronic regions are transcribed differentially.

The complete repertoire of coding and non-coding transcriptome is still under consideration. It is also seen that most transcripts are not poly-adenylated which represents a quite distinct class of sequences some of which are associated with developmental processes. About 95% of transcription sites in humans are not associated with mRNAs. These transcription sites are associated mainly with non-coding transcriptome. These non poly-adenylated transcripts are still highly uncharacterized due to the reason of use of poly-A tails in removing overhangs in rRNA contamination in history.

**What actually long noncoding RNAs are?**

Long noncoding RNAs can be described as any RNAs (noncoding) of having length which about greater than 200 nucleotides excluding all known classes of siRNAs. Any transcript which lack open reading frame and having no codon conservation can be called as non-coding. Recent studies gives strong evidences that proteins are not encoded by most annotated long non-coding RNAs. In experimental studies and databases these long non coding transcripts can be added into intronic or antisense subclasses. Despite of all these, still there is no clue of any intrinsic difference between non-coding and coding genes. For example, in their

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association with activating of compressing chromatic complexes.

**Functions of long non-coding RNAs:**

The surprising invent of eukaryotic large non-coding RNAs some of which are about hundreds of kilo-bases, starts discussion and debate about the function of these RNAs, particularly many of them shows little evolutionary conservation and low expression levels, led to the possibility that long non-coding RNAs represents transcriptional noise having no significance from the biological point of view. However, by various studies it is now clear that long non-coding RNAs from ultra-conserved species to those that are primate specific, shows a variety of evolutionary conservation. Infact there are various strong evidences which proves that although long non-coding RNAs lack somewhat sequence conservation, but it does not lack any function. It is now also confirmed that lncRNAs shows structural conservation. In addition to these conservations long non-coding RNAs also conserved all Bona fide genes which includes promotes, chromatin indicative structure and transcription factors conservation (fig#3). LncRNAs and mRNAs have the same cellular half-lives. Different studies shows the expression ofLong noncoding RNAs in various differentiation systems such as muscles, embryonal stem cells, breast, erythroid as well as neural differentiation. They are also expressed in various diseases such as cancer. To date the function of long non-coding RNA validation depends on knock-down of candidate long non-coding RNAs. The mall interference RNA mediated approaches gives us an easy way of lncRNAs knock-down and also helps to detect phenotypic changes in cells. By now about ~50 lncRNAs are experimentally confirmed and hundreds are published and various are en-route to publication that shows that these long non-coding RNAs are functional.

**The Epigenetic role of regulatory RNA:**

Till now many studies suggests that main function of regulatory RNAs is in epigenetic process regulation. Such as by guiding modifying enzymes of chromatin to their target sites or can act as scaffolding for the organization of chromosomes (fig#3). Firstly, in plants, human and fungi, RNAs were shown to induce gene silencing. It was also observed at that time that small RNAs associates with poly-comb and Ago proteins occurs in the nucleus from where they are involved in the epigenetic process. If we look back to 1990, antisense RNAs again primarily in plants and animals play a role in gene expression. Alternate splicing is controlled with the help of lncRNAs similar to snRNAs.

Long non-coding RNAs may also have the role in the orchestrating the spatial chromatin structure during the process of differentiation and development. This explains the cell specific expression nature of lncRNAs. Many long non-coding RNAs represents the characteristics of enhancers. Such RNAs plays a role in guiding the physical looping between enhancers. They also control transcription and alternate splicing by targeting promoters and exons. A large proportion of lncRNAs have roles in other cellular processes such as protein localization regulation, mRNA translation and synthesis as indicated by the dynamically shuttling to cytoplasm.

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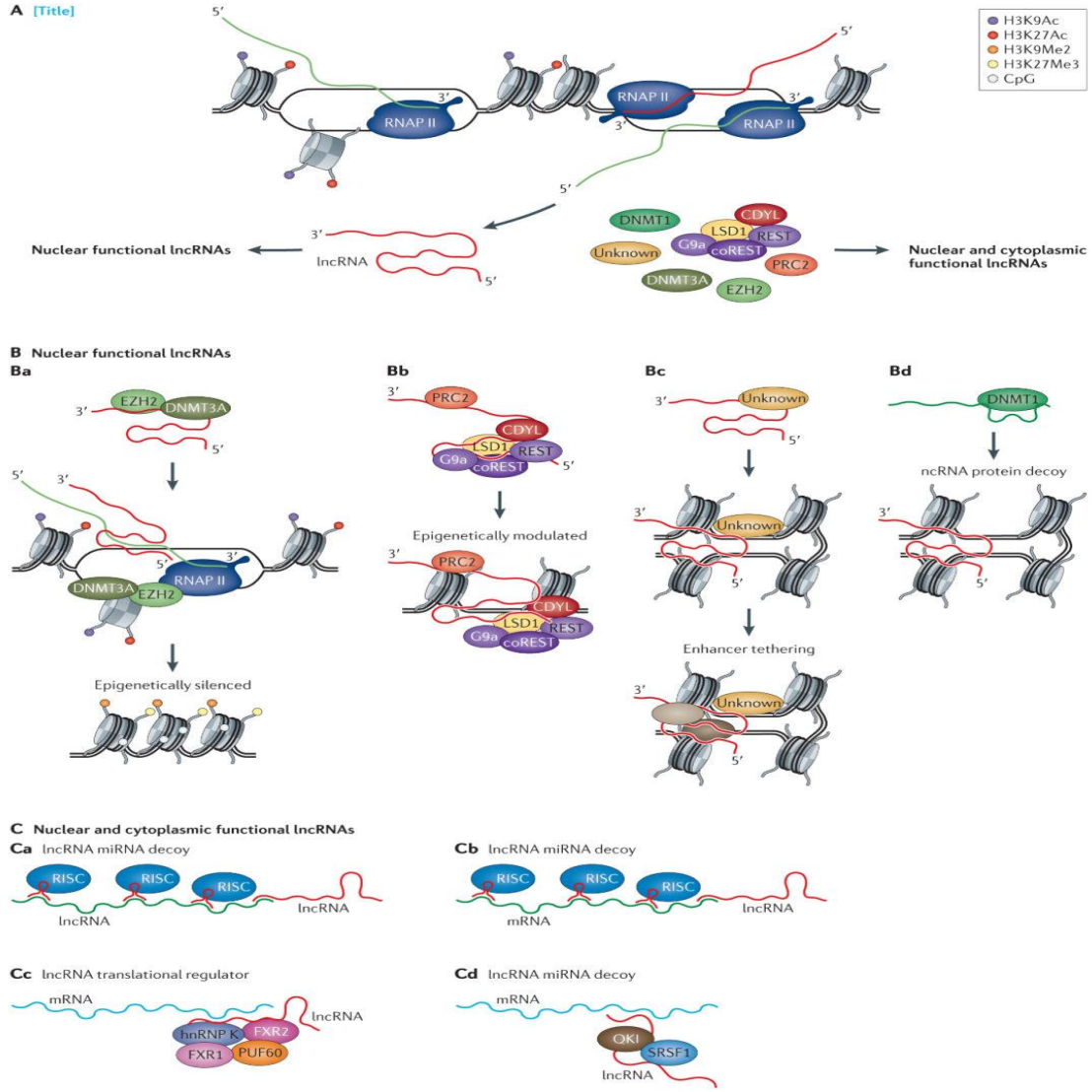


Figure 3 various functions of lncRNAs

**Editing/modification and inheritance of RNA:**

Environmental factors imparts influence on regulatory RNAs which may be transferred between both cells & generations. This influence has the necessary inference for knowing environment and evolution of genes. Various studies suggest that flexibility on RNA is imposed by directed epigenetics with editing of RNA specifically during the course of cognitive evolution and also by the utilization and mobility of retrotransposon. Gene duplication and transposition are the raw materials for evolution. Infact many IncRNAs may arise from retrotransposons and retro transposition may accelerate the evolution of mRNA. Apart from these there are various 100 different modifications of RNAs which are well documented such as adenosine or cytosine methylation. There is a strong evidence of the NRA mediated inheritance in animals and plants.

## II. CONCLUSION:

The past two decades proves to be an effective in understanding the hidden roles and existence of many classes of RNAs. Indeed in the past we were misunderstood the actual genetic programming nature in complex and multicellular organisms. This was due to the wrong presumption that much of genetic information is encoded in polypeptides. To some extent, this assumption was true, but in terms of simpler organisms. Because in complex organisms, the whole genome appears to be dominated by RNAs. There is a very complex

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function as well as evolutionary interaction between protein encoding and non-coding RNAs. Now it is though that some long non-coding RNAs may be developed from protein encoding genes and that capacity of encoding new proteins may also become a part of lncRNAs. The number and diversity of RNAs shows that still we are far apart from the exact understanding of the structure as well as the function of interconnected systems. Still there may be a large number of non-coding RNAs whose function is still unknown, and we have to do a deep sequencing and study to understand them. RNA can fold into complexes and different structures that may have effect on proteins and RNA, DNA guiding by making duplex or triplex conformations because it is not a linear component. There are still many questions to be answered such as about the identification of functional domains in RNA and their interacting patterns after which we will be able to understand the actual functional diversity of RNAs. Infact RNA appears to be the computational engine of cell, molecular, developmental biology, not only this, but for evolution it-self. Now-a-days the main focus in molecular biology is to explore the vast universe of RNA which is still unknown. Indeed, without knowing and understanding the RNA universe, we are unable to understand what actually biology is?

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