Non-Coding RNAs: A Dynamic and Complex Network of Gene Regulation

Munshi A[1], **Mohan V**[2,3*] **and Ahuja YR**[2]

[1]Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad-500016, India

[2]Department of Genetics and Molecular Medicine, Vasavi Medical and Research Centre, Khairatabad, Hyderabad 500004, India

[3]Research Unit, Hansgene Cancer Foundation, Hyderabad, 500004, India

[*]**Corresponding author:** Vasavi Mohan, Vasavi Medical and Research Centre, Department of Genetics and Molecular Medicine, Hyderabad-500004, India, Tel: 91-40-23210251; E-mail: greenpastures@gmail.com

Abstract

It has been estimated that less than two percent of the mammalian genome encodes proteins, rest of the genome which was earlier considered as junk DNA is the treasure trove of non-coding RNAs (ncRNAs). Many ncRNAs have now been characterized. They constitute one of the largest families of gene regulators that are found in plants and animals. They form a complex network and have key roles in diverse regulatory pathways involved in human health and disease. In this review, different types of ncRNAs, their biogenesis, structure, function and evolutionary significance is showcased.

Introduction

Non-coding RNAs (ncRNAs) gained international attention in 1998, when the ability of a double stranded RNA to silence gene expression in *Caenorhabditis elegans* was discovered. For the discovery of such small ncRNAs that interfered with gene expression (RNAi), Andrew Fire and Craig Mellow won the Nobel Prize in Medicine and Physiology in 2006.

Intermediate sized (50 nt - 500 nt) non-coding infra-structural RNAs have been known for a long time and include tRNAs, rRNAs, small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs). These are involved in translation and splicing, and function by sequence-specific recognition of RNA substrate and also in catalysis. In addition to their infra-structural roles, some of these may also have regulatory roles [1]. Many additional ncRNAs have now been characterized and amongst these, are small ncRNAs such as small interfering RNA (siRNAs) (19 nt - 21 nt), microRNAs (22 nt - 25 nt) and piwi-interacting RNAs (26 nt - 31 nt) involved in epigenetically regulating gene expression. Some other small RNAs which have not been studied adequately, have also been described in plants and other lower organisms. They include ta-siRNA (trans-acting siRNA), hc-siRNA (heterochromatic siRNA), scnsRNA (small scan RNA), and qisRNA (QDE2-interacting small RNA) [2]. In addition, several long ncRNAs, ranging from 0.5 kb to over 100 kb, have been shown to regulate gene expression by modifying chromatin structure.

A large proportion of eukaryotic transcription is bidirectional, producing ncRNAs that can overlap with the transcription of protein-coding genes. They interact *inter se* as well as compete for a target on the mRNA. LncRNAs may regulate gene expression via their interaction with other RNA and regulate mRNA stability [3]. They also interact with DNA and proteins to form a complex network that can regulate gene activity with almost infinite potential complexity [4,5]. Certain chromosomal regions contain many regulatory sites that can activate gene expression over long distances and others that counteract

this activity [6]. Furthermore, their activity may not be restricted to the same cell; some ncRNAs may spread to other cells or nuclei by diffusion [7], whereas others may have their activity restricted to specific cell types. The types and functions of different ncRNAs have been summed up in Figure 1.
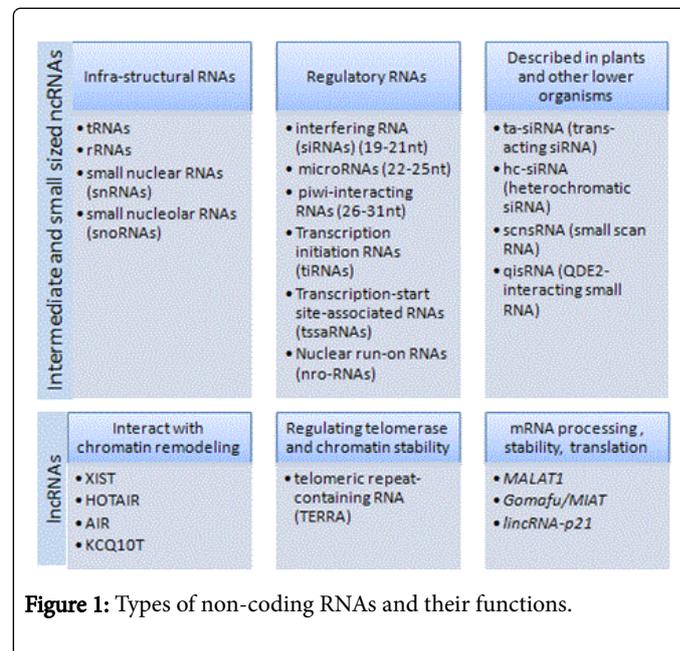

Figure 1: Types of non-coding RNAs and their functions.

Small ncRNAs

Small interfering RNA (siRNA / RNAi)

According to Grosshans and Filipowicz [8], small RNAs are generally produced by fragmentation of longer precursors. Dicer cleaves the precussor dsRNA into shorter (about 20 nt long) double-stranded siRNAs. One siRNA-strand then assembles into an RNA-

induced silencing complex (RISC). The main components of RISC complex are proteins of the Argonaute (Ago) family; Ago2 is the sole enzyme capable of endonucleolytic cleavage [9]. The siRNA in this complex then identifies the mRNA based on sequence complementarity. RISC then cleaves the mRNA in the middle and the resulting mRNA halves are degraded by other cellular enzymes. This mechanism of RNA mediated gene silencing is found in plants more often than in animals [10].

Small interfering RNAs are not only formed from introns of messenger RNAs (mRNAs) but can also be formed from non-coding centromeric DNA transcripts and, in particular, from long terminal repeats of transposons [11]. Small interfering RNAs are regulatory molecules that, besides protecting cells from intrusion of any exogenous nucleic acid (like viruses), are involved in maintaining genomic integrity by silencing transcription from undesired loci like retrotransposons and other repeats [12,13] demonstrated that the RNAi pathways along with directed histone modifications also regulate the organization of the nucleolus in Drosophila.

RNAi and heterochromatin: Heterochromatin is essential for normal chromosomal organization [14], as well as centromere and telomere function [15,16]. In addition, it functions to silence gene expression, reduce the frequency of recombination, promote long range chromatin interactions and ensure accurate chromosome segregation during mitosis [17,18]. Given the functional relevance of heterochromatin and the newer findings, terming non-coding DNA as 'junk' is passé. Pal-Bhadra et al. [19] investigated the mechanism of heterochromatin silencing in Drosophila and noticed that, in addition to DNA methylation and histone modifications, RNAi machinery had a significant role to play in it.

RNAi and centromere behaviour: The centromere is of vital importance for genetic stability. It is the DNA region which ensures separation of chromosomes in mitosis and meiosis. Defects in chromosome segregation are associated with human disease. For example, defects in meiotic chromosome segregation may lead to the production of aneuploid embryos with too few or too many chromosomes [20], and mitotic chromosome segregation errors may contribute to tumor formation [21]. Centrosomal behaviour is governed by epigenetic mechanisms; a centromeric histone variant, CenH3, also known as centromere protein-A (CENP-A), and other histone modifications play key roles at centromeric chromatin in determining centromere identity and kinetochore assembly. In addition, many CENP-A-interacting proteins and factors that affect its localization have been identified [22]. It has been suggested that ncRNA derived from centromeric repeats plays an active role, mostly through the RNAi pathway, in the formation of pericentromeric and centromeric heterochromatin, where both of them are important for proper centromere function [23].

MicroRNAs

Genes for microRNAs are located on all chromosomes and are mostly found within stretches of DNA between clusters of genes, in introns of non-coding or coding genes, as well as in exons of non-coding genes [24,25]. Many microRNAs are transcribed and regulated independently, and use their own transcription initiation regions [26]. These long primary transcripts of microRNA genes (pri-microRNAs) are subsequently cleaved by Drosha (an RNAse III endonuclease) to produce a stem-loop structured precursor (pre-microRNA) about 70 nt long. Pre-microRNAs, each with a short hairpin structure, are delivered by Exportin-5 (Exp5) through the nuclear pores to the cytoplasm [27], where they are processed by Dicer, which chops long dsRNAs into ~ 22 nt duplexes of mature microRNAs. In their role in the maturation of microRNAs, both Drosha and Dicer are associated with a number of co-factors or accessory proteins, with some playing an important regulatory function. For example, Dicer-interacting proteins are helpful in unwinding dsRNAs and loading one strand of the ds-microRNA onto the effector complex, microRNA-induced silencing complex (microRISC). Thermodynamic stability of the 5' end of the duplex determines which of the two strands is retained in the RISC as guide-strand, and which is the passenger-strand that is cut and degraded. Finally, the guide-strand confers specificity to the RISC that now recognizes mRNA targets that are in turn either degraded or translationally repressed [28]. Human microRNAs are typically expressed at high levels (1000-30,000 copies per cell), and can have profound impact on cellular physiology [29].

Although microRNAs are known to mediate post-transcriptional gene silencing in the cytoplasm, recent evidence suggests that at least some fraction of mammalian microRNAs may also activate or inhibit gene repression at the transcriptional level in the nucleus [30,31]. Mostly, microRNAs negatively regulate post-transcriptionally their targets depending on the degree of complementarity between ncRNA and the target. MicroRNA mediated translational repression is considered to be by deadenylation [32]. Nucleotides 2-8 of the mature microRNA sequence create the seed region that primarily identifies the specific mRNA which the microRNA will bind to. Multiple microRNAs bind to cognate sites in the 3' UTR of target RNAs to regulate the protein levels [33,34]. Although the protein levels of these genes are reduced, the mRNA levels of these genes are barely affected. This mechanism of microRNA-mediated gene expression control is mostly seen in animals [35].

MicroRNAs and their targets seem to form complex regulatory networks. For example, a simple microRNA can bind to and regulate many different mRNA targets and, conversely, several different microRNAs can bind to and cooperatively control a single mRNA target [36]. By coordinating and regulating many genes, microRNAs are well-suited to act as stabilizers of gene expression networks and to prevent extreme variations in phenotype due to intrinsic and extrinsic disturbances. The interactions of microRNAs with their mRNA targets with their short signature sequences make them ideal for the combined effects with other microRNAs or RNA-binding proteins (RBPs) that associate with the same mRNA. In addition to conventional 3` UTR targets of mRNAs, [37] have reported targets occurring throughout some mRNAs. For example, mouse transcription factors Nanog, Pouf5f1 / Oct4 and Sox2 display many naturally occurring microRNA targets in their amino acid coding sequence. Now evidence has emerged that in moss (Physcomitrella patens) microRNAs can also silence gene expression at the transcriptional level by interacting with DNA, leading to methylation [38]. This mechanism may well be applicable to other organisms. In fact, many examples have been described of microRNAs regulating their own transcription through single negative or double negative (or positive) feedback loops with specific transcription factors [39].

Although, most of the microRNAs have been implicated in gene silencing, either fully or partially, they are also involved in the transcriptional activation and co-activation of many genes [40]. In other words, microRNAs collectively fine-tune gene expression. Obviously, when a microRNA is misexpressed, it has the potential to interact with targets that might not be a part of its normal endogenous function, dysregulate them, leading to complex diseases [41].

The number of known functional microRNA genes has been estimated to be ~ 1100 [42], a number comparable to those of transcription factors or RNA binding proteins (RBPs), and over one-third of all known human genes are probably regulated by them [43]. Whereas, some microRNAs are ubiquitously expressed, others have an expression pattern that depends on the developmental stage or on the cell type [44]. For example, microRNAs in the gametes may have direct role in the differentiation and development of the early zygote or may play a part in post-fertilization epigenetic reprogramming. Among their other roles, around 70% of microRNAs are expressed in the brain where their expression has been shown to vary dynamically, both before and after birth, indicating a requirement for different microRNAs at different time-points [45]. As a matter of fact, microRNAs, which are expressed in the mammalian brain at different levels, seem to be critical in dictating neuronal cell identity, synaptic development, neuronal plasticity and also affecting learning and memory [46].

MicroRNAs have an important role in the dynamic interplay between the environment and genome, including both genetic and epigenetic processes [47]. For instance, microRNAs can direct the cytosine methylation and histone modifications that are implicated in chromatin modifications [48]. They may control epigenetic notations of specific regions of genome that governs the precision with which DNA methylation and histone modifications occur [49]. Not only that, gene expression has been seen to change by specific environmental stress including chemicals like arsenic, cadmium and aluminium, through specific microRNAs resulting in a specific disease [50].

Signal transduction pathways are prime candidates for microRNA mediated regulation. Emerging evidence suggests that microRNAs affect the responsiveness of cells to signaling molecules such as TGF-β (transforming growth factor-β), WNT, Notch and EGF (endodermal growth factor). MicroRNAs act as inhibitors of proteins mediating the insulin / IGF1 and target of rapamycin (TOR) signaling, both of which are conserved modulators of an organism's life-span [51]. As such, microRNAs serve as nodes of signaling to ensure homeostasis [52]. Abnormal expression of microRNAs can disrupt signaling network in the cells, resulting in pathological changes.

MicroRNAs take part to regulate cell activity by generating pathways parallel to others already present in order to support the signaling processes or provide reinforcement. This kind of action can be exemplified by MiR-34a/b/c activity. This family of microRNAs participates in the execution of p53-dependent senescence, apoptosis and tumor suppressor activity [53,54]. Mutated or inappropriately expressed microRNAs are involved in most human cancers, obesity, diabetes, hair loss, brain disease, skeletal muscle injury and premature senescence [55-57]. In short, microRNAs have key roles in diverse regulatory pathways, including control of metabolism, immunity, developmental timing, cell proliferation, cell differentiation, organ development, senescence and apoptosis.

MicroRNA levels change over the life of an individual and are associated with the aging process [58]. For example, microRNA let-7b is involved in decline of neuronal stem cell self-renewal during aging by reducing HMGA2 levels in old but not in young mice [59,60]. Depletion of Dicer which is involved in the biogenesis of microRNAs, in human cells leads to a significant enhancement of Ataxin-3-induced toxicity, which has been linked to neurodegeneration [61]. MicroRNAs may be contributing factors in neurodegeneration leading to Parkinson disease and Alzheimer disease [29,62]. Post-mortem brain studies of schizophrenics have revealed changes in the expression of certain proteins involved in synaptic neurotransmission and development. Changes in the expression of these proteins seem to be due to alterations in the levels of miR-181b and miR-219 in the cortex [63,64]. Since, emerging evidences suggests that microRNAs play significant roles in the production, action and secretion of insulin and also in diverse aspects of glucose and lipid metabolism, altered levels of microRNAs are critical in the development and progression of diabetes as well as in diabetic complications such as nephropathy, retinopathy and cardiac hypertension [65].

MicroRNAs and paramutation: Paramutation is the epigenetic transfer of information from one allele of a gene to another, without sequence change, to establish a state of gene expression that is inherited. Paramutation has been found at several loci in maize, and at fewer loci in other species, including mice and humans [66]. MicroRNAs contribute to the mechanism of this trans-generational inheritance, establishment and maintenance of paramutations [7,67].

MicroRNAs and epistasis: Epistasis is defined as non-additive genetic interaction; the interaction may be transgressive if the hybrid progeny is either superior to the better or inferior to the worse parent. The mechanism of transgressive segregation is not well understood. After observing positive transgressive segregation (hybrid vigor) in a cross between cultivated tomato *(Solanum lycopersicum)* and one of its wild relatives *(S. pennelli),* Shiveprasad et al. [68] investigated the role of ncRNAs in its mechanism. They observed that the stable transgressive phenotypes in the progeny were associated with small RNAs (microRNA generated from the miR395 allele and siRNAs, a small fraction of loci, 153) which were more abundant in hybrids than in either parent. They proposed that, at least in part, small RNA loci of tomato exhibit transgressive activity, which in turn leads to epigenetic and gene expression changes within hybrid progeny.

MicroRNAs and their mobility: A key feature of microRNAs is their ability to spread from cell to cell. Small RNA mobility is highly regulated both developmentally and in response to physiological and environmental change. Such non-cell autonomous gene repression has been characterized extensively in both plants and animals. However, the precise identity of the mobile silencing signal of these microRNAs remains unsettled. It will be interesting to learn whether mobility is a general property of all microRNAs or restricted to a defined subset [69].

MicroRNAs and telomeres: Vertebrate TTAGGG DNA tandem repeats in telomeres are organized into a heterochromatic structure but remain unmethylated due to lack of methylable cytosine. In contrast subtelomeres are heavily methylated through the action of the DNA methyletransferases [70]. As has already been seen, microRNAs are potential regulators of cellular senescence; they mediate a tight control of DNA methylation that is crucial for telomere homeostasis [71]. Studies have described the role of senescence-associated microRNAs (SA-microRNAs) involved in regulating cellular signaling and cell cycle pathways and directly affecting replicative sencescence (telomere attrition) [72].

MicroRNAs and cancer: Many of the target mRNA transcripts, which are post-transcriptionally regulated by microRNAs, are involved in cell proliferation, differentiation and apoptosis processes commonly altered during tumorigenesis [73]. Reduced levels of certain microRNAs have been seen in cancer cells in comparison to normal cells [74] and this reduction could be due to reduced expression of Drosha or Dicer. For example, reduced expression of Dicer has been seen to be associated with a poor prognosis in lung cancer [75]. Again,

through the analysis of human and mouse model of B-cell lymphoma, it was shown that the prominent consequence of oncogenic over-activation of Myc lead to wide-spread repression of microRNA expression by the binding of Myc to microRNA promoters. When the expression of repressed microRNA was strengthened, it diminished the tumorigenic potential of lymphoma cells [76].

Some microRNAs have emerged as candidate components with oncogenic function and some others as tumor suppressor regulators. For example, miR 372-373 has been implicated as proto-oncogene in testicular cancer [77] and let-7 has shown a potential as tumor suppressor in various cancers [78]. Loss of let-7 correlates with over-expression of Ras proteins which are oncogenic [79]. Recent studies have shown that miR-34 family is direct transcriptional target of p53. MiR-34 activation can mimic p53 activities, including induction of cell-cycle arrest and promotion of apoptosis. Loss of miR-34 can impair p53 mediated cell death [53].

MicroRNAs may play a dual role, as oncogenes or anti-oncogenes. For example, in one context, the c13orf25 cluster microRNAs act as an oncogene; in another context, it seems to antagonize the effects of different oncogenes, acting like a classic tumor suppressor gene. Variability created by SNPs in the binding sites of microRNAs and their target sites on mRNA, along with other surrounding influences due to genetic and epigenetic architecture, may determine the role of a particular microRNA [80]. In human cancer, microRNA expression can be altered by several other mechanisms: chromosomal abnormalities, mutations and polymorphisms (SNPs), defects in microRNA biogenesis and epigenetic changes like altered DNA methylation and histone deacetylase inhibition [81]. Apart from exposure to irradiation and chemotherapy during treatment, non-genic causes in the tumor lead to stresses such as hypoxia and nutrient deficiency. Stress has been seen to cause relocation of Argonaute family members within the cell [82]. The association of Argonaute with other RNA binding proteins recently has been shown to switch a normally repressive microRNA into an activator of its target, when cells are under stress due to starvation of serum or amino acids [28,83]. Sorting out the microRNA regulatory networks is going to be a real challenge.

Many of the mechanisms of epigenetic control such as DNA methylation and histone modifications, known to regulate protein-coding genes, also seem to be applied to microRNA genes. Many advanced tumors show defects in microRNA expression and processing which could increase phenotypic variability within tumors. This allows small subsets of cells with altered characteristics to emerge, which can have grave consequences since typically a small fraction of tumor cells is responsible for metastasis and treatment resistance, and ultimately treatment failure. Many microRNAs are found in CpG islands, and it is likely that mirSNPs in CpG islands also affect the pattern of microRNA expression and contribute to cancer susceptibility, response to treatment and prognosis. Although candidate gene approaches can certainly ascertain the effect of single SNP on an individual risk of cancer, the cumulative effect of the inheritance of multiple SNPs in microRNA-related genes might augment risk. Consistent with this idea, an increased risk of esophageal and bladder cancer was observed in individuals with SNPs in both microRNAs and microRNA processing genes [84]. MicroRNA markers might indicate the initial risk of cancer, and predict those patients at higher risk of post-surgical recurrence. For example, [85] observed that miR-106a and miR-148a expression correlated with post-surgical recurrence of esophageal cancer and tumor related mortality.

More than 50% of microRNA genes are located in fragile sites and common breakpoint regions frequently associated with cancer [86]. B-cell chronic lymphocytic leukemia (CLL), which is most common adult leukemia in developed countries, is associated with over-expression of the anti-apoptotic oncogenic protein Bcl-2. CLL is often associated with the loss of chromosomal region 13q14 [87], and within this deleted region are the transcription sites of miR-15a and miR-16-1 which seem to inhibit the Bcl-2 protein activity [88]. It has been demonstrated in animal models that if misregulated microRNAs are restored to their normal state, it can bring partial or full recovery from diseases like cancer [89].

Solid tumors (> 1 mm – 2 mm) need neovasculature to remove metabolic waste and provide oxygen and nutrients, an important step in the neoplastic transformation. The extent of new vascularization may be proportional to the metastatic potential of the tumor. miR-126 is highly expressed during embryonic development and in endothelial cells, has been implicated promoting angiogenic processes; targeted deletion of this microRNA resulted in defective vascularization and embryonic death in both mice and zebrafish [62]. MicroRNAs (miR-17–92 cluster, miR-221, miR-222), that target other factors like anti-angiogenic protein Thrombospondin 1 and inhibit endothelial cell migration as well as proliferation, also have been identified.

MicroRNAs may have differential expression in different ethnic populations. In the USA, prostate cancer affects Afro-American males at a much higher rate than the Caucasian males. No target genes have been identified so far for this difference. A microarray study on 10 Caucasians and 8 Afro-Americans showed significantly over-expressed as well as down-regulated microRNAs in the Afro-Americans compared with the Caucasian individuals. The list of microRNAs that were at least three times differentially expressed included miR-301, miR-26a, miR-1b-1 and miR-30c-1 [87].

MicroRNAs produced by viruses: Viral miRNAs are reported to regulate expression of viral as well as host genic expression by interfering with the repression or cleavage of mRNA transcripts, also thereby influencing host cellular processes that respond to viral infection. The herpesvirus ICP34.5 protein promotes replication of the virus in neuronal cells *in vivo*. miR-I derived from HSV-1 has been shown to reduce the protein expression level of ICP34.5 in HSV-1-infected cells [90]. Hypothesized that the control of ICP34.5 expression in individual infected neurons by these microRNAs may affect their virulence or latency in the host. Another miRNA expressed by HSV-1 prevents apoptosis by blocking the expression of two host cellular proteins, SMAD3 and transforming growth factor-β [91]. Epstein-Barr virus-encoded microRNA miR-BART2 down-regulates the levels of aberrant BALF5 mRNA transcripts in order to prevent viral replication during latency [92]. MiR-BART-1-5p, miR-BART-16 and miR-BART-17-5p were shown to downregulate EBV latent membrane protein 1 (LMP1) through recognition of the 3'UTR of its mRNA, thus providing a role for miRNAs in establishment of latent infection and promoting host cell survival. EBV also expresses ncRNAs that interfere with host cellular interferon responses.

Micrornas and transgenerational inheritance: The mechanism by which the epigenome is transmitted via mitosis seems to be through the role of DNA methyltransferase (DNMTs) and histone deacetylases (HDACs) [93]. Its transmission between different generations of organisms is a subject of considerable controversy as it was traditionally thought that epigenetic marks were cleared through the process of meiosis. More recent studies suggest that microRNAs

transmitted through meiosis can restore the state of the epigenome in zygote [7].

Piwi-interacting RNAs

Piwi-interacting RNAs (piRNAs) are derived from various repetitive elements of the genome, such as centromeric and telomeric heterochromatin, and are very rich in sequences cognate to all classes of transposable elements. piRNA genes exist in the genome in clusters; individual clusters range between 1 kb and 100 kb in size and encode between 10 piRNAs and 4500 piRNAs. Although bidirectional clusters are known, the majority of piRNA clusters are monodirectional, i.e., within a given cluster all piRNAs are derived from one of the two strands of DNA [2,94]. piRNAs are generated from long single–stranded transcripts in a process independent of Drosha and Dicer; the processing enzyme has not been identified yet. These small RNAs (26 nt - 31 nt) associate with subfamily of Argonaute proteins called Piwi proteins, which are mainly germline specific. Several thousand piRNAs have been identified, and together with their Piwi partners, they are essential for the development of germ cells [95]. Piwi proteins are also expressed in somatic cells that are in close contact with germline cells. Consistent with their expression pattern and function, Piwi mutant animals exhibit germ cell developmental defects such as azoospermia and tesicular tumors [96,97]. piRNAs mediate de novo methylation and histone modification machinery in silencing transposable element sequences in the mammalian male germ line [98]. Approximately one million piRNA molecules have been reported per spermatocyte or round spermatid [99]. Although the exact function and targets of piRNAs are unclear, piRNAs ensure genomic stability in the germline by silencing selfish genetic elements like transposons by heterochromatin formation [96,100].

Retrotransposable elements comprise around 50% of the mammalian genome. piRNAs are produced from inactive transposable elements through a ping-pong mechanism and form a surveillance system against active transposable element invasion [100] and silence their activities by antisense targeting their mRNA. piRNAs can significantly increase the fitness of organisms by reducing the fitness of retrotransposons. However, target retrotransposons also have higher probability of reaching high frequency of fixation in a population because their deleterious effects are considerably attenuated [101].

Some other small RNAs

Small nucleolar RNAs (snoRNAs) are another class of small RNAs and contribute to RNA modifications of ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs) and other RNAs [49] Emerging evidence suggests that small nuclear ncRNAs may be involved in the regulation of alternative splicing [102,103].

Transcription initiation RNAs (tiRNAs) are short ncRNAs of ~ 18 nt length. They are associated with highly expressed genes and may be implicated in the control of their expression [104]. Transcription-start site-associated RNAs (tssaRNAs) are transcribed in flanking regions of active promoters of protein-coding genes where they may help to maintain an active state [105].

Nuclear run-on RNAs (nro-RNAs) were identified as a group of small RNAs that are active in human promoters associated with RNA polymerase II [106]. These short ncRNAs may also have a function in promoter activation and transcription orientation.

A recently discovered class of non-coding RNAs in the fungus *Neurospora crassa* are qiRNAs named after their interaction with the Argonaute protein QDE-2. Most of them are derived from the rRNA locus, and their transcription is induced by DNA damage. There are indications that qiRNAs might be involved in DNA repair [107].

Long Non-coding RNAs

Transcription actually occurs essentially everywhere, including both coding regions and non-coding regions, and often on both strands [108,109]. It has been reported that changes in expression of these lncRNAs is associated with both development and disease [110]. Recently, [111] have shown the interplay between the expression of two long ncRNAs, transcribed on opposite strands, which can exert epigenetic metastatic control on the transcription of the adjacent protein-encoding FLOW11 gene in yeast *(Saccharomyces cerevisiae)*. This regulatory mechanism has a profound effect on the life cycle of yeast. When FLOW11 is on, diploid cells grow into filaments called pseudohyphae, and haploid cells invade the agar when grown in plates; on the other hand when FLOW11 is off, neither of these events occurs and the cells grow in their familiar budding pattern.

Current estimates of long ncRNAs in the human genome range from ~ 7000-23000. Long ncRNAs are involved in cellular signaling networks associated with human stem cell differentiation [112] Long ncRNAs exihibit cell-type specific repression localized to specific subcellular compartments [113]. Genes once silenced by inclusion into the silent domains of the long ncRNAs are capable of reactivation in a tissue or developmental stage-specific manner by enhancers which are also tissue and development stage-specific [114]. Under adverse environmental conditions, long ncRNAs may lead to disease. For example, recent work on Alzheimer disease has identified a ~ 2 kb ncRNA, which is induced in response to numerous cell stresses, and which increases the stability of the BACE1 mRNA, thus leading to even more Aβ-peptides involved in neuronal damage. In a nutshell, they are associated with human health and disease [115].

Long ncRNAs can act on domains ranging in size from a single promoter to an entire chromosome, and they can function in *cis* or *trans* to establish chromatin conformations which either activate or repress transcription [116]. It is now established that some long ncRNAs such as XIST, HOTAIR, AIR and KCQ10T interact with chromatin remodeling complexes targeting them to specific genes to exert their functions [117]. Molecular functions of long ncRNAs include modulating transcriptional patterns, regulating protein activities, serving structural or organizational roles, altering RNA processing events, and serving as precursors to small RNAs [118]. In mammals, transcription of long ncRNAs has been shown to contribute to various processes including T cell receptor recombination [119] and maintenance of telomeres. Long ncRNA transcripts, called telomeric repeat-containing RNA (TERRA), are involved in regulating telomerase and chromatin stability [120]. Long ncRNAs are also involved in genomic imprinting and X-chromosome inactivation. The ncRNAs as described to be produced by some viruses are hundreds to thousands of nucleotides in length and may have the ability to regulate gene expression in favor of the viral genome [121]. There are examples of long and short ncRNAs intersecting with each other and lncRNAs can themselves be host genes for small RNAs, for example H19 is host to microRNA-675 [122].

Long ncRNAs and enomic imprinting

Genomic imprinting is a *cis* acting epigenetic process by which a subset of autosomal genes is not expressed in a parent of origin-specific manner. In mammals approximately 100 such genes clustered in 25 regions have been identified [123]. A typical imprinted cluster consists of 2-12 protein coding genes, and at least one long ncRNA, which is usually expressed from the maternal allele and invariably shows bidirectional reciprocal expression to the coding genes.

A key feature of imprinted gene clusters is the presence of an imprint control element (ICE). The ICE is epigenetically modified only on one parental chromosome by a DNA methylation 'imprint', which is acquired during maternal or paternal gametogenesis and is maintained on the same chromosome in the diploid embryo [124]. The ICE carries histone modifications that are specific to the DNA-methylated allele, i.e., repressive histone marks are associated with the DNA-methylated ICE, whereas active histone marks are associated with the unmethylated ICE [125].

Imprinted gene expression is controlled by differential DNA methylation of an ICE, which is read by an epigenetic initiator that silences genes in the surrounding imprinted gene cluster in *cis*. Imprinted genes close to the epigenetic initiator tend to show ubiquitous imprinted expression, whereas genes further away are involved in showing placental specific expression [126].

The ICE methylation imprint is universal, present in all tissues and all stages of development (except germ cells), whereas imprinted impression is not always present and may vary during development, differentiation and disease. For example, in post-mitotic neurons, imprinted expression of IGF2 is lost and it shows biallelic expression [127]. Similarly, many cases of human colorectal cancer are associated with loss of imprinted expression that results in biallelic IGF2 expression [128].

Long ncRNAs and x-chromosome inactivation

X-chromosome inactivation in female mammals is epigenetic dosage compensation by an unknown mechanism. For the inactivation of X-chromosome the 17 bp long non-coding Xist RNA (inactive X-specific transcript RNA), which operates in *cis,* is essential [129]. X-inactivation centre (XIC) requires the expression and spread of the Xist ncRNA over one of the X-chromosomes to induce a cascade of chromatin changes that ultimately result in transcriptional repression of over 1000 X-linked genes [130], leaving 15% of the X-linked genes to be expressed [131]. These changes include incorporation of the histone variant macroH2A, DNA methylation and recruitment of PcG (polycomb group) proteins. These chromatin changes allow the inactivated X-chromosome to be stably silenced at later stages of development, even in the absence of Xist [132]. Tsix (inactive X-specific transcript), a 40 kb ncRNA transcript with antisense orientation of Xist, acts as a negative modulator of Xist expression by blocking Xist RNA accumulation along the future active X-chromosome [133]. In other words, long ncRNAs are not only limited to regulating the expression of protein-coding genes, but also are involved in regulating the expression of other long ncRNAs. Moreover, it is interesting to note that long ncRNAs are also involved in inactive X-chromosome perinuclear localization [134].

For random X inactivation, a particular system has evolved that relies on the large ncRNA Xist that is repressed from and localizes specifically to the inactive X chromosome (Xi). Xist expression is controlled by a mechanism that ensures that one X chromosome remains active in a diploid cell. Regulation involves sequences around the Xist gene, which makes up the complex genetic locus of the XIC. Xist RNA has been identified in human and mouse and apparently conserved among placental mammals [135].

Long ncRNAs and trans-splicing events

For some genes the epigenome harbors a 'splicing code' that determines tissue specific splicing outcome [136]. Some long non-coding RNAs may be involved in *trans*-splicing events in certain genes. For example, CDC2L2 gene located on chromosome 1 is associated with fine-tuning of the cell division and apoptotic activities in testis [137]. Recently, it has been shown that CDC2L2 mRNA is *trans*-spliced by a ncRNA transcript from Y-chromosome. In the long arm of Y-chromosome there is a major heterochromatic block, and there is a consensus sequence of GGAAT in this block which is transcribed specifically in the testis. A long ncRNA transcript from Yq12 in the heterochromatic blocks trans-splices CDC2L2 mRNA from chromosome 1p36.3 locus to generate a testis specific chimeric βsr13 isoform of CDC2L2. This trans-splicing event could be involved in regulating the activity of CDC2L2 in testis [138].

Long ncRNAs and cancer

Similar to protein-coding oncogenes, long ncRNAs (e.g. HOTAIR) can also promote cellular pathways that lead to tumorigenesis. HOTAIR is expressed from HOX locus, and it is overexpressed in breast cancer. Enforced expression of HOTAIR results in an altered pattern of H3K27 methylation and increased invasiveness, whereas the depletion of HOTAIR causes the opposite cellular phenotype. Furthermore, HOTAIR expression level in primary breast tumors is a powerful predictor of patient's outcomes such as metastasis and death [139].

Recent studies have identified numerous long ncRNAs that are induced by the p53 tumor suppressor pathway [140]. When cells are subjected to stress the transcription factor p53 initiates a tumor suppressor program that involves the expression and repression of many genes. In particular, one of this long ncRNA-p21, which is required for the global repression of genes that interfere with p53 function, regulates cellular apoptosis [141].

In the near future, the great technological advances and decrease in cost of parallel massive sequencing will allow the profiling of the entire transcriptome of every type of tumor, including small and long ncRNA molecules, allowing the most powerful and informed diagnosis [142].

Long ncRNAs and paraspeckles

Paraspeckles are ribonucleoprotein bodies in the inter-chromatin space of mammalian cell nuclei. One polyadenylated long ncRNA occurs exclusively in these speckles [113]. A number of RNA–binding proteins, including paraspeckle–protien-complex-1, together with the RNA-NEAT-1, form parapeckles [143]. Paraspeckles are not present in human embryonic stem cells but only appear upon differentiation [144]. These bodies are ~ 0.5 μm - 1.0 μm in size, and their number varies both within cell populations depending on cell type. These structures play a role in regulating the expression of certain genes in differentiated cells [145].

ncRNAs and RNA Granules

In higher organisms, granules that contain protein, mRNAs and ncRNAs are found in the cytoplasm of somatic and germ cells. Specific components of these RNA granules can alter DNA and RNA sequences, and can regulate transcription in a form of cytoplasmic inheritance [146]. For example, in cloning when a somatic nucleus is transferred to an enucleated egg, the cytoplasmic RNA granules present in this egg reprogram the somatic nucleus to a state of totipotency so that embryogenesis can get started in the clone.

During mitosis, germ-cell granules shrink and disappear from the part of the cell that will become the soma, and fuse or enlarge in the part of the cell that will remain germline [147].The differentiation of somatic and germline daughter cells is determined in part by the absence or presence of germ-cell granules. These are also acquired to specify the germline in the next generation, which represents clear example of epigenetic inheritance [146].

Diagnostic and Therapeutic Potential of ncRNAs

The cell cycle is a tightly orchestrated process during normal development. MicroRNAs seem to play a central role in achieving this process. Manipulating the expression of this large family of cell-cycle-regulating microRNAs may provide an important therapeutic avenue. Emerging evidence suggests that expression profiling of microRNAs may be used in diagnostics [148]. Different cancer types have distinct microRNA profiles. Accurately predicting microRNA targets for any known microRNA will provide a useful tool to accelerate the progress of microRNA studies in pathology and cancer developmental biology.

Unlike mRNAs, endogenous microRNAs can be robustly and reliably measured in sputum or plasma / serum over days of storage or freeze-thawing cycles remaining largely intact [149]. Also, small RNAs can easily be measured from the formalin fixed tissue specimens used routinely in hospital pathology laboratories. Hence, the potential of microRNA based diagnostics could fit simply into the standard hospital workflow. In some cases, epigenetic mechanisms are responsible for silencing microRNA expression in cancer. MicroRNA methylation signatures could be useful in diagnosis and prognosis of cancer [150].

Therapeutic interventions based on manipulation of microRNA levels could also be put to use as a novel approach for treating the genetic diseases [151]. For example, modified antisense oligonucleotides regulate the expression of microRNAs in the same way as classical tumor suppressor genes [152]. MicroRNA-based therapy could also be useful to enhance sensitivity to conventional drugs used in cancer treatment. MicroRNAs are emerging as predictors and modifiers of chemo- and radio-therapy in different tumor types [85]. The delivery of a synthetic let-7 mimic induced remission of established non-small cell lung carcinoma in mice. Effective silencing of miR-122 using inhibitors with locked nucleic acid antimiR and cholesterol conjugation showed long-lasting decrease in plasma cholesterol in primates, with no evidence of treatment-related toxicity. The results from these studies indicate the potential of this microRNA-based therapeutics in the clinical setting.

RNAi based therapies in viral diseases seem promising and are picking up interest [153]. Endogenous cellular microRNAs that target viral RNAs have been reported as well. The siRNAs have been used to decrease the drug resistance of cells *in vitro* by inhibiting the repression of MDR1, a multidrug resistance complex [154]. The

drawbacks of siRNA include off-target effects, elicitation of the interferonresponse and their interference with microRNA biogenesis, hampering its use as a therapeutic. microRNAs are exempt from these problems with added features of specificity and their potential for selective multiple targeting, make them the technology of choice for intervention [155]. MicroRNA mimicry has recently been used *in vitro* where functional assays can be performed to identify cellular processes and phenotypic changes associated with specific microRNAs transfected into cell lines.

Keeping in view the above mentioned possibilities, several preclinical and clinical trials have been initiated for microRNA based therapeutics [156].

Evolutionary Significance of ncRNAs

An overwhelming evidence for the diverse roles the ncRNAs play in gene expression suggests that they are indeed the architects of eukaryotic complexity from the evolutionary point of view [4]. A large number of ncRNAs identified so far depict highly conserved sequences within the animal and plant kingdoms. However, a great deal of divergence occurred between the two kingdoms. A comparative analysis has shown that the complexity of organisms is inversely proportional to the protein coding genes in their genomes. In fact, the ncRNA-mediated gene regulation is widespread among higher eukaryotes, when compared to prokaryotes. In humans, about 98 percent of the total transcriptional output is non-coding. The complex genetic phenomena like interference, gene silencing, imprinting, co-suppression, methylation, acetylation, position effect related variegation, transvection and paramutation are hypercyclically interconnected pathways through which ncRNA signalling is affected.

Nc-RNAs like SRA / SRAP may function both as non-coding regulatory sequences and as an mRNA transcript for protein synthesis. From an evolutionary point of view, they may actually be 'protogenes' where we have evidence from literature that previously non-genic sequences may evolve into protein-coding genes [157].

The paradigm shift in gene expression and regulation needs to be examined transcending the classical central dogma of molecular biology. Perhaps, the ncRNAs have evolved to enable the integration and networking of complex suits of gene-action to constitute a second tier gene-expression among eukaryotes. The proportion of ncRNA has linearly increased during evolution to suit the emergence of complex organisms of eukaryota [158].

ncRNAs and Future Challenges

There are many challenges that researchers have to resolve. For example, delivery of the molecules to the right cells is a technical hurdle. Some efforts have been made to overcome this hurdle. For example [159] described the ability of mesenchymal stem cell to migrate directly to breast tumors, after effectively bypassing immune surveillance; these stem cells could be used to deliver antisense microRNA strands to the target and reduce off-target effects of microRNA knockdown. Furthermore, many individual microRNAs home in on dozens or even hundreds of genes. It is also important to identify all the genes individual microRNAs influence to ensure that modifying their expression will not have untoward effects. It will also be desirable to identify specific environmental, lifestyle and dietary exposures that alter the expression of ncRNAs and consequently their respective gene targets.

Another challenge will be to confirm functional capacity of any ncRNA to alter the expression of target genes. This is complicated by the fact that ncRNAs do not contain any protein coding information that would allow distinguishing the functional ncRNAs species from pseudo-ncRNAs. Until now, the functional ncRNA species have mostly been validated *in vitro* using luciferase reporter activity; however, further development of the strategies to facilitate the identification of functional ncRNAs is needed [160]. Moreover lncRNAs may be involved in regulating the transcriptome and not mere 'transcriptional noise' anymore. There may be a plethora of molecular functions they may be involved in, which need further investigation.

Acknowledgement

References

1. Gibney ER, Nolan CM (2010) Epigenetics and gene expression. Heredity (Edinb) 105: 4-13.

2. Choudhuri S (2009) Lesser known relatives of miRNA. Biochem Biophys Res Commun 388: 177-180.

3. Szcześniak MW, Makałowska I (2016) lncRNA-RNA Interactions across the Human Transcriptome. PLoS One 11: e0150353.

4. Mattick JS, Gagen MJ (2001) The evolution of controlled multitasked gene networks: the role of introns and other noncoding RNAs in the development of complex organisms. Mol Biol Evol 18: 1611-1630.

5. Ebert MS, Sharp PA (2010) Emerging roles for natural microRNA sponges. Curr Biol 20: R858-861.

6. Ruf S, Symmons O, Uslu VV, Dolle D, Hot C, et al. (2011) Large-scale analysis of the regulatory architecture of the mouse genome with a transposon-associated sensor. Nat Genet 43: 379-386.

7. Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, et al. (2006) RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. Nature 441: 469-474.

8. Grosshans H, Filipowicz W (2008) Molecular biology: the expanding world of small RNAs. Nature 451: 414-416.

9. Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, et al. (2004) Argonaute2 is the catalytic engine of mammalian RNAi. Science 305: 1437-1441.

10. Tang G, Reinhart BJ, Bartel DP, Zamore PD (2003) A biochemical framework for RNA silencing in plants. Genes Dev 17: 49-63.

11. Hennig W (2004) The revolution of the biology of the genome. Cell Res 14: 1-7.

12. Naqvi AR, Islam MN, Choudhury NR, Haq QM (2009) The fascinating world of RNA interference. Int J Biol Sci 5: 97-117.

13. Peng JC, Karpen GH (2007) H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. Nat Cell Biol 9: 25-35.

14. Dernburg AF, Sedat JW, Hawley RS (1996) Direct evidence of a role for heterochromatin in meiotic chromosome segregation. Cell 86: 135-146.

15. Bernard P, Maure JF, Partridge JF, Genier S, Javerzat JP, et al. (2001) Requirement of heterochromatin for cohesion at centromeres. Science 294: 2539-2542.

16. De Lange T (2005) Telomere-related genome instability in cancer. Cold Spring Harb Symp Quant Biol 70: 197-204.

17. Jia S, Yamada T, Grewal SI (2004) Heterochromatin regulates cell type-specific long-range chromatin interactions essential for directed recombination. Cell 119: 469-480.

18. Pidoux AL, Allshire RC (2004) Kinetochore and heterochromatin domains of the fission yeast centromere. Chromosome Res 12: 521-534.

19. Pal-Bhadra M, Leibovitch BA, Gandhi SG, Rao M, Bhadra U, et al. (2004) Heterochromatin silencing and HP 1 localization in Drosophila are dependent on the RNAi machinery. Science 303: 669-672.

20. Lamb NE, Hassold TJ (2004) Nondisjunction--a view from ringside. N Engl J Med 351: 1931-1934.

21. Weaver JC, Alessio AC, Brown SE, Hellstorm IC, Dymov S, et al. (2007) The transcription factor nerve-growth factor-induced protein which mediates epigenetic programming: Altering epigenetic marks by immediate early genes. J Neurosci 27: 1756-1768.

22. Allshire RC, Karpen GH (2008) Epigenetic regulation of centromeric chromatin: old dogs, new tricks? Nat Rev Genet 9: 923-937.

23. Pezer Z, Ugarković D (2008) Role of non-coding RNA and heterochromatin in aneuploidy and cancer. Semin Cancer Biol 18: 123-130.

24. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A (2004) Identification of mammalian microRNA host genes and transcription units. Genome Res 14: 1902-1910.

25. Schanen BC, Li X (2011) Transcriptional regulation of mammalian miRNA genes. Genomics 97: 1-6.

26. Song, G, Wang L (2008) MiR-433 and miR-127 arise from independent overlapping primary transcripts encoded by the miR-433-127 locus. PLoS One 3: 3574.

27. Yi R, Qin Y, Macara IG, Cullen BR (2003) Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 17: 3011-3016.

28. Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W (2006) Relief of microRNA-Mediated translational repression in human cells subjected to stress. Cell 125: 1111-1124.

29. Nelson PT, Wang WX, Rajeev BW (2008) MicroRNAs (miRNAs) in neurodegenerative diseases. Brain Pathol 18: 130-138.

30. Kim DH, Saetrom P, Snøve O Jr, Rossi JJ (2008) MicroRNA-directed transcriptional gene silencing in mammalian cells. Proc Natl Acad Sci U S A 105: 16230-16235.

31. Turner AM, Morris KV (2010) Controlling transcription with noncoding RNAs in mammalian cells. Biotechniques 48: ix-xvi.

32. Djuranovic S, Nahvi A, Green R (2011) A parsimonious model for gene regulation by miRNAs. Science 331: 550-553.

33. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB (2003) Prediction of mammalian microRNA targets. Cell 115: 787-798.

34. Wu W, Sun M, Zou GM, Chen J (2007) MicroRNA and cancer: Current status and prospective. Int J Cancer 120: 953-960.

35. Lim LP, Lau LN, Engelle GP, Grimson A, Schelter J, et al. (2005) Microarray analysis shows that some microRNAs down regulate large numbers of target mRNAs. Nature 433: 769–773.

36. Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120: 15-20.

37. Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I (2008) MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455: 1124-1128.

38. Khraiwesh B, Arif MA, Seumel GI, Ossowski S, Weigel D, et al. (2010) Transcriptional control of gene expression by microRNAs. Cell 140: 111-122.

39. Krol J, Loedige I, Filipowicz W (2010) The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 11: 597-610.

40. Goodrich JA, Kugel JF (2009) From bacteria to humans, chromatin to elongation, and activation to repression: The expanding roles of noncoding RNAs in regulating transcription. Crit Rev Biochem Mol Biol 44: 3-15.

41. Farh KK, Grimson A, Jan C, Lewis BP, Johnston WK, et al. (2005) The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. Science 310: 1817-1821.

42. Sato F, Tsuchiya S, Meltzer SJ, Shimizu K (2011) MicroRNAs and epigenetics. FEBS J 278: 1598-1609.

43. Davalos V, Esteller M (2010) MicroRNAs and cancer epigenetics: a macrorevolution. Curr Opin Oncol 22: 35-45.

44. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, et al. (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129: 1401-1414.

45. Lewis MA, Steel KP (2010) MicroRNAs in mouse development and disease. Semin Cell Dev Biol 21: 774-780.

46. Presutti C, Rosati J, Vincenti S, Nasi S (2006) Non coding RNA and brain. BMC Neurosci 7 Suppl 1: S5.

47. Mattick JS, Amaral PP, Dinger ME, Mercer TR, Mehler MF (2009) RNA regulation of epigenetic processes. Bioessays 31: 51-59.

48. Costa FF (2005) Non-coding RNAs: new players in eukaryotic biology. Gene 357: 83-94.

49. Mattick JS, Makunin IV (2005) Small regulatory RNAs in mammals. Hum Mol Genet 14 Spec No 1: R121-132.

50. Patel CJ, Butte AJ (2010) Predicting environmental chemical factors associated with disease-related gene expression data. BMC Med Genomics 3: 17.

51. Grillari J, Grillari-Voglauer R (2010) Novel modulators of senescence, aging, and longevity: Small non-coding RNAs enter the stage. Exp Gerontol 45: 302-311.

52. Inui M, Martello G, Piccolo S (2010) MicroRNA control of signal transduction. Nat Rev Mol Cell Biol 11: 252-263.

53. He L, He X, Lim LP, de Stanchina E, Xuan Z, et al. (2007) A microRNA component of the p53 tumour suppressor network. Nature 447: 1130-1134.

54. Kumamoto K, Spillare EA, Fujita K, Horikawa I, Yamashita T, et al.(2008) Nutlin-3a activates p53 to both down-regulate inhibitor of growth and up regulate mir-34a, mir-34b and mir-34c expression, and induce senescence. Cancer Res 68: 3193-3203.

55. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, et al. (2005) A microRNA polycistron as a potential human oncogene. Nature 435: 828-833.

56. Mudhasani R, Zhu Z, Hutvagner G, Eischen CM, Lyle S, et al. (2008) Loss of miRNA biogenesis induces p19Arf-p53 signaling and senescence in primary cells. J Cell Biol 181: 1055-1063.

57. Yang Z, Wu J (2007) MicroRNAs and regenerative medicine. DNA Cell Biol 26: 257-264.

58. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, et al. (2007) Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. Cell 129: 303-317.

59. Nishino J, Kim I, Chada K, Morrison SJ (2008) Hmga2 promotes neural stem cell self-renewal in young but not old mice by reducing p16Ink4a and p19Arf Expression. Cell 135: 227-239.

60. MuÃ±oz-Najar U, Sedivy JM (2011) Epigenetic control of aging. Antioxid Redox Signal 14: 241-259.

61. Bilen J, Liu N, Burnett BG, Pittman RN, Bonini NM (2006) MicroRNA pathways modulate polyglutamine-induced neurodegeneration. Mol Cell 24: 157-163.

62. Wang SC, Oelze B, Schumacher A (2008) Age-specific epigenetic drift in late-onset Alzheimer's disease. PLoS One 3: e2698.

63. Beveridge NJ, Tooney PA, Carroll AP, Gardiner E, Bowden N, et al. (2008) Dysregulation of miRNA 181b in the temporal cortex in schizophrenia. Hum Mol Genet 17: 1156-1168.

64. Kocerha J, Faghihi MA, Lopez-Toledano MA, Huang J, Ramsey AJ, et al. (2009) MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. Proc Natl Acad Sci USA 106: 3507–3512.

65. Pandey AK, Agarwal P, Kaur K, Datta M (2009) MicroRNAs in diabetes: tiny players in big disease. Cell Physiol Biochem 23: 221-232.

66. Chandler VL (2007) Paramutation: from maize to mice. Cell 128: 641-645.

67. Cuzin F, Grandjean V, Rassoulzadegan M (2008) Inherited variation at the epigenetic level: paramutation from the plant to the mouse. Curr Opin Genet Dev 18: 193-196.

68. Shiveprasad PV, Dun RM, Santos BA, Bassett A, Baulcome DC (2012) Extraordinary transgressive phenotypes of hybrid tomato are influenced by epigenetics and small silencing RNAs. EMBO J 31: 257-266.

69. Chitwood DH, Timmermans MC (2010) Small RNAs are on the move. Nature 467: 415-419.

70. Brock GJ, Charlton J, Bird A (1999) Densely methylated sequences that are preferentially localized at telomere-proximal regions of human chromosomes. Gene 240: 269-277.

71. Benetti R, Gonzalo S, Jaco I, Muñoz P, Gonzalez S, et al. (2008) A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rb12-dependent regulation of DNA methyltransferases. Nat Struct Mol Biol 15: 268-279.

72. Lafferty-Whyte K, Cairney CJ, Jamieson NB, Oien KA, Keith WN (2009) Pathway analysis of senescence-associated miRNA targets reveals common processes to different senescence induction mechanisms. Biochim Biophys Acta 1792: 341-352.

73. Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T, et al. (2007) Impaired MicroRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 39: 673–677.

74. Lee EJ, Baek M, Gusev Y, Brackett DJ, Nuovo GJ, et al. (2008) Systematic evaluation of microRNA processing patterns in tissues, cell lines, and tumors. RNA 14: 35-42.

75. Karube Y, Tanaka H, Osada H, Tomida S, Tatematsu Y, et al. (2005) Reduced expression of Dicer associated with poor prognosis in lung cancer patients. Cancer Sci 96: 111-115.

76. Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, et al. (2008) Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet 40: 43-50.

77. Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, et al. (2006) A genetic screen implicates miRNA372 and miRNA373 as oncogenes in testicular germ cell tumors. Cell 124: 1169–1181.

78. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, et al. (2005) RAS is regulated by the let-7 microRNA family. Cell 120: 635-647.

79. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, et al. (2006) Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 9: 189-198.

80. Fabbri M, Valeri N, Calin GA (2009) MicroRNAs and genomic variations: from Proteus tricks to Prometheus gift. Carcinogenesis 30: 912-917.

81. Iorio MV, Croce CM (2009) MicroRNAs in cancer: small molecules with a huge impact. J Clin Oncol 27: 5848-5856.

82. Leung AK, Calabrese JM, Sharp PA (2006) Quantitative analysis of Argonaute protein reveals microRNA-dependent localization to stress granules. Proc Natl Acad Sci U S A 103: 18125-18130.

83. Vasudevan S, Steitz JA (2007) AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. Cell 128: 1105-1118.

84. Ye Y, Wang KK, Gu J, Yang H, Lin J, et al. (2008) Genetic variations in microRNA-related genes are novel susceptibility loci for esophageal cancer risk. Cancer Prev Res (Phila) 1: 460-469.

85. Hummel R, Hussey DJ, Haier J (2010) MicroRNAs: predictors and modifiers of chemo- and radiotherapy in different tumour types. Eur J Cancer 46: 298-311.

86. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, et al. (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancer. Proc Natal Acad Sci USA 101: 2999–3004.

87. Calin GA, Croce CM (2007) Chromosome rearrangements and micro RNAs: a new cancer link with clinical implications. J Clin Invest 117: 2059-2066.

88. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, et al. (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 102: 13944-13949.

89. Vicki G, 2008 Tapping miRNA-regulated pathways. Genet Eng Biotechnol News 28.

90. Tang S, Bertke AS, Patel A, Wang K, Cohen JI, et al. (2008) An acutely and latently expressed herpes simplex virus 2 viral microRNA inhibits

expression of ICP34.5, a viral neurovirulence factor. Proc Natl Acad Sci USA 105: 10931-6.

91. Gupta A, Gartner JJ, Sethupathy P, Hatzigeorgiou AG, Fraser NW (2006) Anti-apoptotic function of a microRNA encoded by the HSV-1 latency-associated transcript. Nature 442: 82-85.

92. Barth S, Pfuhl T, Mamiani A, Ehses C, Roemer K, et al. (2008) Epstein-Barr virus-encoded microRNA miR-BART2 down-regulates the viral DNA polymerase BALF5. Nucleic Acids Res 36: 666-675.

93. Rountree MR, Bachman KE, Baylin SB (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. Nat Genet 25: 269-277.

94. Zamore PD s(2010) Somatic piRNA biogenesis. EMBO J 29: 3219-3221.

95. Grosshans H, Filipowicz W (2008) Proteomics joins the search for microRNA targets. Cell 134: 560-562.

96. Aravin AA, Sachidanandam R, Girard A, Fejes-Toth K, Hannon GJ (2007) Developmentally regulated piRNA clusters implicate MILI in transposon control. Science 316: 744-747.

97. Esteller M (2011) Non-coding RNAs in human disease. Nat Rev Genet 12: 861-874.

98. Siomi MC, Sato K, Pezic D, Aravin AA, (2011) PIWI-interacting small RNAs: the vanguard of genome defence. Nat Rev Mol Cell Biol 12: 246-258.

99. Aravin A, Gaidatzis D, Pfeffer S, Lagos-Quintana M, Landgraf P, et al. (2006) A novel class of small RNAs bind to MILI protein in mouse testes. Nature 442: 203-207.

100. Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, et al. (2007) Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. Cell 128: 1089-1103.

101. Lu J, Clark AG (2010) Population dynamics of PIWI-interacting RNAs (piRNAs) and their targets in Drosophila. Genome Res 20: 212-227.

102. Khanna A, Stamm S (2010) Regulation of alternative splicing by short non-coding nuclear RNAs. RNA Biol 7: 480-485.

103. Luco RF, Allo M, Schor IE, Kornblihtt AR, Misteli T (2011) Epigenetics in alternative pre-mRNA splicing. Cell 144: 16-26.

104. Taft RJ, Pheasant M, Mattick JS (2007) The relationship between non-protein-coding DNA and eukaryotic complexity. Bioessays 29: 288-299.

105. Seila AC, Calabrese JM, Levine SS, Yeo GW, Rahl PB, et al. (2008) Divergent transcription from active promoters. Science 322: 1849-1851.

106. Core LJ, Waterfall JJ, Lis JT (2008) Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science 322: 1845-1848.

107. Lee HC, Chang SS, Choudhary S, Aalto AP, Maiti M, et al. (2009) qiRNA is a new type of small interfering RNA induced by DNA damage. Nature 459: 274-277.

108. Berretta J, Morillon A (2009) Pervasive transcription constitutes a new level of eukaryotic genome regulation. EMBO Rep 10: 973-982.

109. Mercer TR, Dinger ME, Mattick JS (2009) Long non-coding RNAs: insights into functions. Nat Rev Genet 10: 155-159.

110. Kung JT, Colognori D, Lee JT (2013) Long noncoding RNAs: past, present, and future. Genetics 193: 651-669.

111. Bumgarner SL, Dowell RD, Grisafi P, Gifford DK, Fink GR (2009) Toggle involving cis-interfering noncoding RNAs controls variegated gene expression in yeast. Proc Natl Acad Sci U S A 106: 18321-18326.

112. Kikuchi K, Fukuda M, Ito T, Inoue M, Yokoi T, et al. (2009) Transcripts of unknown function in multiple-signaling pathways involved in human stem cell differentiation. Nucleic Acids Res 37: 4987-5000.

113. Sone M, Hayashi T, Tarui H, Agata K, Takeichi M, et al. (2007) The mRNA-like noncoding RNA Gomafu constitutes a novel nuclear domain in a subset of neurons. J Cell Sci 120: 2498-2506.

114. Saxena A, Carninci P (2011) Long non-coding RNA modifies chromatin: epigenetic silencing by long non-coding RNAs. Bioessays 33: 830-839.

115. Prasanth KV, Prasanth SG, Xuan Z, Hearn S, Freier SM, et al. (2005) Regulating gene expression through RNA nuclear retention. Cell 123: 249-263.

116. Whitehead J, Pandey GK, Kanduri C (2009) Regulation of the mammalian epigenome by long noncoding RNAs. Biochim Biophys Acta 1790: 936-947.

117. Ponting CP, Oliver PL, Reik W (2009) Evolution and functions of long noncoding RNAs. Cell 136: 629-641.

118. Wilusz JE, Sunwoo H, Spector DL (2009) Long noncoding RNAs: functional surprises from the RNA world. Genes Dev 23: 1494-1504.

119. Abarrategui I, Krangel MS (2007) Noncoding transcription controls downstream promoters to regulate T-cell receptor alpha recombination. EMBO J 26: 4380-4390.

120. Luke B, Lingner J (2009) TERRA: telomeric repeat-containing RNA. EMBO J 28: 2503-2510.

121. Swaminathan S (2008) Noncoding RNAs produced by oncogenic human herpesviruses. J Cell Physiol 216: 321-326.

122. Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, et al. (2012) The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. Nat Cell Biol 14: 659-665.

123. Williamson CM, Blake A, Thomas S, Beechey CV, Hancock J, et al. (2009) World wide web site-mouse imprinting data and references. MRC Harwell: Oxfordshire.

124. Mohammad F, Mondal T, Kanduri C (2009) Epigenetics of imprinted long noncoding RNAs. Epigenetics 4: 277-286.

125. Koerner MV, Pauler FM, Huang R, Barlow DP (2009) The function of non-coding RNAs in genomic imprinting. Development 136: 1771-1783.

126. Hudson QJ, Kulinski TM, Huetter SP, Barlow DP (2010) Genomic imprinting mechanisms in embryonic and extraembryonic mouse tissues. Heredity (Edinb) 105: 45-56.

127. Yamasaki Y, Kayashima T, Soejima H, Kinoshita A, Yoshiura K, et al. (2005) Neuron-specific relaxation of Igf2 imprinting is associated with neuron-specific histone modifications and lack of its antisense transcript Air. Hum Mol Genet 14: 2511-2520.

128. Kaneda A, Feinberg AP (2005) Loss of imprinting of IGF2: a common epigenetic modifier of intestinal tumor risk. Cancer Res 65: 11236-11240.

129. Chow J, Heard E (2009) X inactivation and the complexities of silencing a sex chromosome. Curr Opin Cell Biol 21: 359-366.

130. Payer B, Lee JT (2008) X chromosome dosage compensation: how mammals keep the balance. Annu Rev Genet 42: 733-772.

131. Carrel L, Willard HF (2005) X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434: 400-404.

132. Chaumeil J, LeBaccon P, Wutz A, Heard E (2006) A novel role for Xist RNA in the formation of a repressive nuclear compartment into which genes are recruited when silenced. Genes Dev 20: 2223-2237.

133. Stavropoulos N, Lu N, Lee JT (2001) A functional role for Tsix transcription in blocking Xist RNA accumulation but not in X-chromosome choice. Proc Natl Acad Sci U S A 98: 10232-10237.

134. Zhang H, Darwanto A, Linkhart TA, Sowers L, Zhang L (2007) Maternal cocaine administration causes an epigenetic modification of protein kinase gene expression in fetal rat heart. Pharmacology 71: 1319-1328.

135. Leeb M, Wutz A (2010) Mechanistic concepts in X inactivation underlying dosage compensation in mammals. Heredity (Edinb) 105: 64-70.

136. Fox-Walsh K, Fu XD (2010) Chromatin: the final frontier in splicing regulation? Dev Cell 18: 336-338.

137. Gururajan R, Lahti M, Grenet J, Easton J, Gruber I, et al. (1998) Duplication of genomic region contaning the Cdc2L1-2 and MMP21-22 genes on human chromosome 1p36.3 and their linkage to D1Z2. Genome Res 8: 929-939.

138. Jehan Z, Vallinayagam S, Tiwari S, Pradhan S, Suresh A, (2007) Novel noncoding RNA from human Y distal heterochromatic block (Yq12) generates testis-specific chimeric CDC2L2. Genome Res 17: 433-440.

139. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, et al. (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 464: 1071-1076.

140. Gutman M, Amit I, Garber M, French C, Lin MF, et al. (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458: 223-227.

141. Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, et al. (2010) A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell 142: 409-419.

142. Huarte M, Rinn JL (2010) Large non-coding RNAs: missing links in cancer? Hum Mol Genet 19: R152-161.

143. Bond CS, Fox AH (2009) Paraspeckles: nuclear bodies built on long noncoding RNA. J Cell Biol 186: 637-644.

144. Chen LiL, Carmichael GG (2009) Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: functional role of a nuclear noncoding RNA. Mol Cell 35: 467-478.

145. Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, et al. (2009) An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Mol Cell 33: 717-726.

146. Anderson P, Kedersha N (2009) RNA granules: post-transcriptional and epigenetic modulators of gene expression. Nat Rev Mol Cell Biol 10: 430-436.

147. Gallo CM, Munro E, Rasoloson D, Merritt C, Seydoux G (2008) Processing bodies and germ granules are distinct RNA granules that interact in C. elegans embryos. Dev Biol 323: 76-87.

148. Calin GA, Croce CM (2006) MicroRNA signatures in human cancers. Nat Rev Cancer 6: 857-866.

149. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, et al. (2008) Circulating microRNAs as stable blood based markers for cancer detection. Proc Natl Acad Sci USA 105: 10513–10518.

150. Lujambio A, Ropero S, Ballestar E, Fraga MF, Cerrato C, et al. (2007) Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res 67: 1424-1429.

151. Marquez RT, McCaffrey AP (2008) Advances in microRNAs: implications for gene therapists. Hum Gene Ther 19: 27-38.

152. Weiler J, Hunziker J, Hall J (2006) Anti-miRNA oligonucleotides (AMOs): ammunition to target miRNAs implicated in human disease? Gene Ther 13: 496-502.

153. Tan FL, Yin JQ (2004) RNAi, a new therapeutic strategy against viral infection. Cell Res 14: 460-466.

154. Nieth C, Priebsch A, Stege A, Lage H (2003) Modulation of the classical multidrug resistance (MDR) phenotype by RNA interference (RNAi). FEBS Lett 545: 144-150.

155. Denby L, Baker AH2 (2016) Targeting non-coding RNA for the therapy of renal disease. Curr Opin Pharmacol 27: 70-77.

156. Wahid F, Shehzad A, Khan T, Kim YY (2010) MicroRNAs: synthesis, mechanism, function, and recent clinical trials. Biochim Biophys Acta 1803: 1231-1243.

157. Tautz D, Domazet-LoÅ¡o T (2011) The evolutionary origin of orphan genes. Nat Rev Genet 12: 692-702.

158. Costa FF (2008) Non-coding RNAs, epigenetics and complexity. Gene 410: 9-17.

159. Dwyer RM, Potter SM, Harrington KA, Lowery AJ, Hennessy E, et al. (2007) Monocyte chemotactic protein-1 (MCP-1) secreted by primary breast tumours stimulates migration of mesenchymal stem cells (MSCs). Clin Cancer Res 13: 5020–5027.

160. Krutovskikh VA, Herceg Z (2010) Oncogenic microRNAs (OncomiRs) as a new class of cancer biomarkers. Bioessays 32: 894-904.