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Existence of snoRNA, microRNA, piRNA characteristics in a novel non-coding RNA: x-ncRNA and its biological implication in *Homo sapiens*

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Comparative genomic analysis constitutes a powerful approach for the systematic understanding of genome. The comparative and evolutionary analysis of known microRNAs in *Homo sapiens* elucidates the discovery of the existence of the novel non-coding RNA, which is transcribed from the unique gene precursor and produces three different non coding RNAs such as piRNA, snoRNA and microRNA. This new characteristics RNA is named as x-non coding RNA (x-ncRNA). This characteristic pattern shows evolutionary signatures dictated by its precise selective significance and synteny. The evidence of x-ncRNA processing and functionality from hairpin arms were proven by systematic analysis. x-ncRNA analysis proven the bio genesis of human piRNA is originated from the dsDNA, which is also involved in the synthesis of piRNA and/or microRNA, through dicer. These data also shows the presence of human sno-microRNA. Our data demonstrates that the existence of x-ncRNA, genomic origin, conceivable bio genesis pathway, evolutionary relationship and its functions.

Key words: Biogenesis, snoRNA, microRNA, piRNA, novel non-coding RNA (x-ncRNA).

INTRODUCTION

Mature microRNAs (miRNA) are short, non-coding ssRNAs (~20 nucleotide) those are repress post-transcriptional mRNA by binding to partial complementary sites, called miRNA binding sites, in their target sites. Mature miRNAs are cleaved from ~70-80 nucleotide hairpin structures, called precursor miRNAs (pre-mRNAs), by the enzyme dicer. Pre-miRNAs are in turn excised from a primary miRNA (pri-miRNA) transcript by the enzyme drosha. Pri-miRNAs are typically transcribed by RNA polymerase II and seem to possess promoter and enhancer elements that are similar to those of protein-coding genes (Biemar et al., 2005; Johnson et al., 2003; Lee et al., 2004). Pre-miRNAs are exported to the cytoplasm, where another RNase III-like enzyme, dicer, liberates a ~20 nt long miRNA duplex from the hairpin.

One strand of the duplex is integrated into an active RNA-induced silencing complex (RISC) (Schwarz et al., 2003), which can be formed, by thousands of nucleotides and contains multiple pre-miRNAs. In some cases, however, pre-miRNAs are contained in introns of protein-coding genes and are excised by splicing machinery. A large number of the human miRNA gene expression (these corresponds to 1% of the protein coding genes) was confirmed, but the predicted miRNA targets remain to be identified and verified. Several bio informatics groups developed algorithms for mRNA identification sequences that could serve as target sites for known miRNAs.

Research groups provided evidence that miRNAs may act as key regulators of processes like early development, cell proliferation and cell death, apoptosis and fat metabolism and cell differentiation. Recent studies of miRNA expression show miRNA implication in brain development, chronic lymphocytic leukemia, colonic adenocarcinoma, Burkitt's lymphoma and viral infection, suggesting possible links between miRNAs and viral disease, neurodevelopment and cancer. There is speculation that in higher eukaryote, the role of miRNAs in regulating

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gene expression could be as important as that of transcription factors (Brennecke et al., 2003; Calin et al., 2004; Krichevsky et al., 2003; Metzler et al., 2004; Michael et al., 2003; Pfeffer et al., 2004; Reinhart et al., 2000; Xu et al., 2003).

Small nucleolar RNAs (snoRNAs) are a class of small RNA molecules (60-300 nts) that guide 2'-O-methylation or pseudouridylation of rRNAs, tRNAs and other snRNAs. The intronic snoRNAs fall into two structurally and functionally well-defined classes that are C/D and H/ACA RNAs. The term small nucleolar RNA was originally coined to reflect the nucleolar localization of the first members of this group relative to their nucleoplasmic cousins, the snRNAs. Most C/D and H/ACA RNAs are involved in rRNA modification and processing in the nucleolus. Intronic snoRNAs can mature by two alternative pathways. They are usually processed from excised and debranched introns by the action of exonucleases that digest away non-snoRNA sequences.

In a second minor pathway, not dependent on splicing, snoRNAs are excised from introns by endonucleases and mature ends are then generated exonucleolytically. Both RNA maturation and nucleolar targeting appear to be mediated by the same RNA structure, the box C/D motif. Since it seems certain that this motif is a protein recognition signal and because of its small size, it seems likely that,

(i) These functions are mediated by the same protein or a small set of core proteins.
(ii) Both motif-dependent processes require formation of the same initial complex.

Specific signals, centred on boxes C and D, and boxes H and ACA, acting as binding sites for snoRNP proteins, are required for snoRNAs faithful processing (Terns and Terns, 2002; Weinstein and Steitz, 1999). To form functional small-nucleolar ribonucleoproteins (snoRNPs), the mature box C/D snoRNAs are associate with fibrillarin, the 15.5 kDa protein, and nucleolar proteins Nop56 and Nop58, whereas the box H/ACA snoRNAs form complexes with dyskerin, Nop10, non-histone chromosome protein 2 (Nhp2) and glycine arginine rich protein 1 (Gar1). Maturation of box C/D snoRNAs can also include,

(i) Modification of internal nucleotides, little is known about (Reddy, 1988; Reddy et al., 1979; Wise et al., 1983).
(ii) Formation of a 5' trimethylguanosine (TMG) cap. TMG cap formation is believed to result from hypermethylation of the m7G cap normally found in RNA polymerase II transcripts. Ongoing studies of snRNPs and snoRNPs revealed unexpectedly elaborate bio genesis pathways.

The Piwi (P-element induced wimpy testis in Drosophila) (Saito et al., 2006) class of genes have been originally identified as encoding regulatory proteins responsible of maintaining incomplete differentiation in stem cells and maintaining the stability of cell division rates in germ line cells (Cox et al., 2000). Three Piwi subfamily proteins - MIWI, MIWI2 and MILI - have been found to be essential for spermatogenesis in mice. Piwi proteins are highly conserved across evolutionary lineages and are present in both plants and animals. The Piwi proteins are part of a protein family called the argonautes. Piwi-interacting RNAs (piRNA) are short stretches of RNAs with a typical length of 26-31 nucleotides. piRNA is a class of small RNA molecules that is expressed uniquely in mammalian testes and forms RNA-protein complexes with Piwi proteins. piRNAs was identified in the genomes of mice, rats and humans, with an unusual "clustered" genomic organization (Girard et al., 2006) that may originate from repetitive regions of the genome such as retrotransposons or regions normally organized into heterochromatin, and which are normally derived exclusively from the antisense strand of double-stranded RNA (Vagin et al., 2006). piRNA has a role in RNA silencing via the formation of a RNA-induced silencing complex (RISC), although the bio genesis of piRNA was not yet clearly understood.

Conservation of microRNA pathway in many species and the utilization of this critical pathway by target species and target gene indicate critical role played in biological system. Target microRNA sequences were harvested by using sangers micrRNA registry. Conserved microRNA targets were analysed to understand the possible conservation that elucidates the new class of x-ncRNA. We coined this name because of the following reasons, indeed, the ongoing discovery of new class of ncRNA (microRNAs, short interfering RNA (siRNAs), repeat-associated RNAs and piRNAs) and new members of existing classes (small nucleolar (sno) RNAs) and the analysis of their biogenesis revealed an unexpected complexity in their assembly, trafficking and mechanisms of action. x-ncRNA is processed from ~86 nucleotide hairpin structures and its function is inside the cytoplasm itself as a snoRNA.

The same precursor gene sequences processed and liberated ~28 nucleotide microRNA and/or piRNA. Since a particular gene transcript was processed and developed as a piRNA, snoRNA and microRNA, we decided to name it x-ncRNA. This kind of genomic cluster is quite unusual. Our data demonstrate the discovery of x-ncRNA and discussion about the possible bio genesis of x-ncRNA in *Homo sapiens*. Bio genesis of x-ncRNA is an unknown mechanism and the understanding of its pathway will require farther affords. The present study was designed to understand the unusual non coding RNA cluster in human.

METHODS

Data bases, Conservation and Evolutionary analysis

To search the microRNAs, miRBase database was used http://micr-

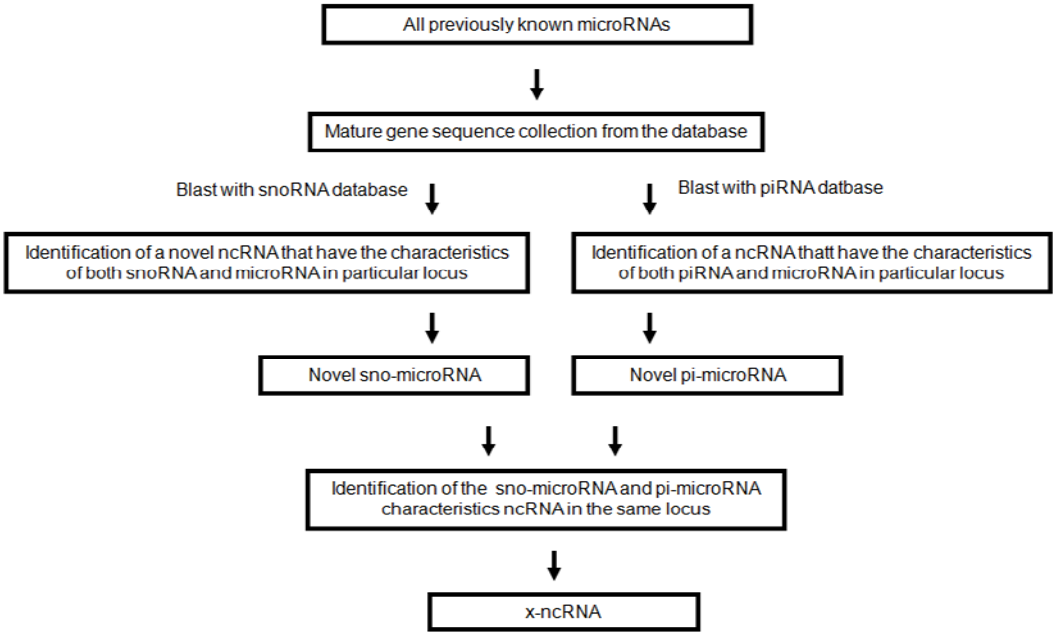


Figure 1. Schematic illustration of methodology for identified human x-ncRNA.

orna.sanger.ac.uk/sequences/). We downloaded all *Homo sapiens* microRNA precursors and mature microRNAs from the microRNA registry. This is an online database of microRNA gene sequences and predicted target sites, version 10.1 of miRBase contains 462 human microRNA gene sequences (Griffiths-Jones, 2004; Griffiths-Jones et al., 2006; Griffiths-Jones et al., 2008), were used for the comparative analysis. To analyze the presence of sno-microRNA, the hoarded human microRNAs, mature microRNAs gene sequences were blasted with snoRNA database (a comprehensive database of human H/ACA and C/D box snoRNAs), version 3 (http://www-snorna.biotoul.fr) (Lestrade and Weber, 2006).

Also these mature gene sequences were blasted with piRNA database (http,//pirnabank.ibab.ac.in/index.shtml) to describe the novel ncRNA that have the characteristics of both piRNA and microRNA in particular locus of the gene. The presence of the sno-microRNA and pi-microRNA has been identified. This sequential analysis were used to identify the novel ncRNA that have the characteristics of snoRNA, microRNA, and piRNA. Using these analytical methods, an x-ncRNA was discovered and its presence in the particular locus were identified (Figure 1). To elucidate its evolutionary conservation and to retrieve the hanging sequences of the x-ncRNA, multiple sequences alignment was performed. Genome blast was used from the National Center for Biotechnology Information (http,//www.ncbi.nlm.nih.goh). The x-ncRNA secondary structure was constructed using Mfold software for the illustration of the loop formation and base pairs complementation.

RESULTS

Gene complements

The cluster location comparison of known microRNA was made with snoRNAs and piRNAs. Totally 462 human microRNAs targets were harvested and these were used to find the overlap of gene sequence to understand the orthologous. For the BLAST analysis precursor and mature sequences were used. The sno-microRNA discovery was made using this analysis, more in detail the result shows that a microRNA (hsa-mir768) was 100% aligned with a snoRNA (SNORD71) (Huttenhofer et al., 2001). The possible clusters of piRNAs with microRNAs were also investigated. Amazingly only one of a mature microRNA was 100% aligned with a piRNA (that is, a pi-microRNA was identified) and this was the same microRNA complementted with snoRNA (that is, a sno-microRNA). This complement is quite unusual and its identification allowed clarifying a new family of non-coding RNA (ncRNA). We indicated this ncRNA as an x-ncRNA, and its genomic origin, evolutionary significance and possible bio genesis were discussed below (Figure 1).

Genomic origin of x-ncRNA

To elucidate the genomic origin of the conserved x-ncRNA, human mature microRNAs precursor sequences were analysed. Since mature microRNA sequences and precursor gene sequence multiple alignments were missing an evolutionary conservation, different methods to understand it by simple BLAST with diverse database were used. All known human mature microRNAs gene sequences were blasted with snoRNA database. More in detail, microRNA gene sequences complementary with sno RNAs were investigated, the full sequence alignment of hsa-mir768 with the SNORD71 using blast was obtained. The SNORD71 in Homo sapiens otherwise called sno RNA HBII-239, small nucleolar RNA and C/D box 71 be-

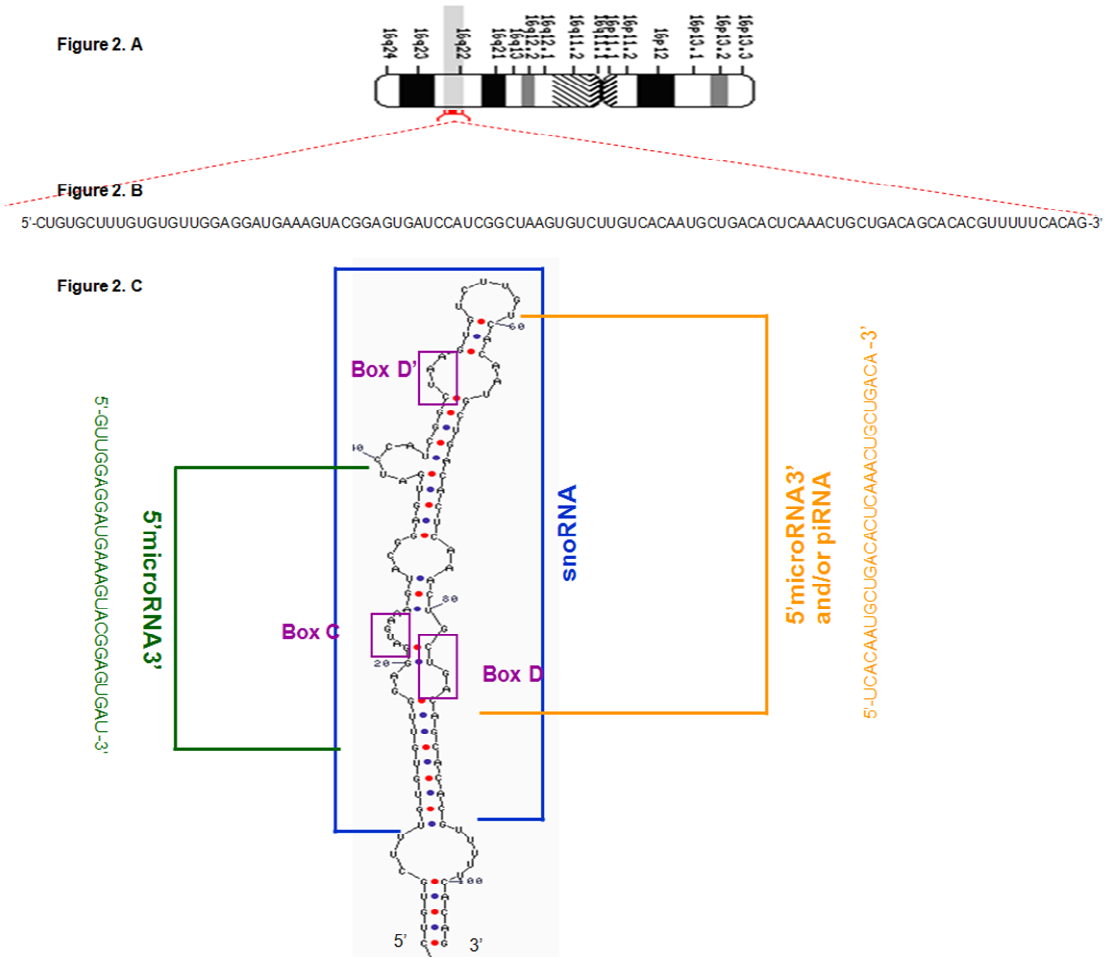


Figure 2. Genomic origin of x-ncRNA:
A. View of chromosome 16q22 on which is present x-ncRNA gene sequence.
B. The gene sequence is the complete sequence of x-ncRNA in 5'-3' position.
C. RNA secondary structure predicted by mfold software using complete sequence of x-ncRNA.
Purple colour boxes indicate the presence of C/D box and also C'/D' box which is evolutionary characteristics of snoRNA. The blue box showing precursor sequence of microRNA and location of 5'-3' mature microRNA is indicated in green colour along with the sequence. The yellow colour indicates the presence of 3'-5' mature microRNA sequence and the same sequence involved as a piRNA, this is also pointed with the gene sequence. The arrow to the sequence indicates the 5'U base of Aub-bound piRNA.

longing to the family of C/D snoRNAs, is present on chromosome 16q22.2 (Figure 2A).

The results show that mature hsa-mir 768 microRNA is present in the SNORD71 gene sequence, besides the SNORD71 not only is mature microRNA gene sequence but also precursor gene sequences. These findings clearly show the presence of sno-microRNA and this is very important to confirm the gene sequence name without making any pitfall on the public database. The precursor gene sequence as follow as, >5'CTGTGCTTTGTGTGTTGGAGGATGAAAGTACGGAGTGATCCATCGGCTAAGTGTCTTGTCACAATGCTGACACTCAAACTGCTGACAGCACACGTTTTTCACAG-3'< (Red coloured sequences are the mature gene sequence). Therefore, we are proposing to indicate this gene as sno-microRNA (Berezikov et al., 2006; Lestrade and Weber, 2006; Li et al., 2007) and further blast analysis with other databases confirmed and elucidated the existence of a novel x-ncRNA.

Briefly all known microRNAs were blasted with most recently discovered piRNA families, only one microRNA overlapped with a piRNA was found. Surprisingly this microRNA is the same which overlapped with snoRNA. The results clearly shows that the presence of sno-microRNA and pi-micrRNA in the same locus of the gene. The name that we propose for this ncRNA is x-ncRNA instead of sno-microRNA, since a unique ncRNA sequence is pro-

cessed as a three different ncRNAs such as piRNA, snoRNA and microRNA. These evidences evoke a new family of small RNA as a x-ncRNA (Figure 2B, C).

Evolutionary analysis of x-ncRNA

The genome comparison of x-ncRNA allowed estimating the homologous dependency of recombination on divergence and distance. x-ncRNA precursor genome blast analysis provided evolutional relationship with Macaca mulatta 98%, Pan troglodytes 97%, Sorex araneus 92% identity (Figure 3A). The analysis of mature gene sequences blasted with total genome elucidates that when Homo sapiens and Macaca mulatta share genes, the DNA sequences in these genes are 100% identical (Figure 3B). Macaca mulatta is the third primate genome to have been sequenced, following those for humans (Homo sapiens) and chimpanzees (Pan troglodytes). The chimpanzee and Rhesus monkeys have a special role in informing studies of human population genetics, a field that is undergoing in rapid expansion and acquiring new relevance to human medical genetics research in neuroscience, behavioural biology, reproductive physiology, endocrinelogy, cardiovascular studies, pharmacology and other area. Nonetheless, identified x-ncRNA suggests that the approach outlined here may help to unlock some of the undisclosed functional characteristics of small RNA families, bio genesis and also the impact of x-ncRNA in human evolution through a combination of within-species and cross-species. Moreover the comparison of the x-ncRNA with the total genome showed that the adenosine nucleotide flanked in front of the 5' uracil nucleotide in Macaca mulatta, Pan troglodytes and also in the Sorex araneus but not in Homo sapiens, although mature sequences start from the 5' uracil nucleotide (Figure 3C). This particular gene shows genomic contig between the human chromosome 16, rhesus monkey chromosome 20 and chimpanzee chromosome 16. These unique syntony might have the evolutionary reason in the genomic origin of human x-ncRNA.

Bio genesis of x-ncRNA

The identified x-ncRNA may involve three plausible bio genesis pathways. The first is the generation of x-ncRNA pathway like snoRNA. The identified sequences having the C/D-box structure were showed to be essential to correct snoRNA synthesis and nucleolar localization (Samarsky et al., 1998). Based on this pathway x-ncRNA produced from the precursor gene sequence and it is processed to act as a snoRNA.

The second pathway, until now not clear, is piRNA bio genesis. The lack of a dependence on dicer and the profound strand asymmetry of mammalian pachytene clusters indicated that piRNA is not generated from the double strand (Houwing et al., 2007; Vagin et al., 2006). In theory, any RNA polymerase such as RNA polymerase II or even primase could produce a piRNA precursor molecule (Bateman and Wu, 2007). Since our results explain that x-ncRNA precursor is involved (as an existed sno RNA and precursor of microRNA) to synthesis the mature sequences to produce mature sequence of piRNA and/or microRNA, and the RNA polymerase is involved in the bio genesis of the x-ncRNA (Figure 4).

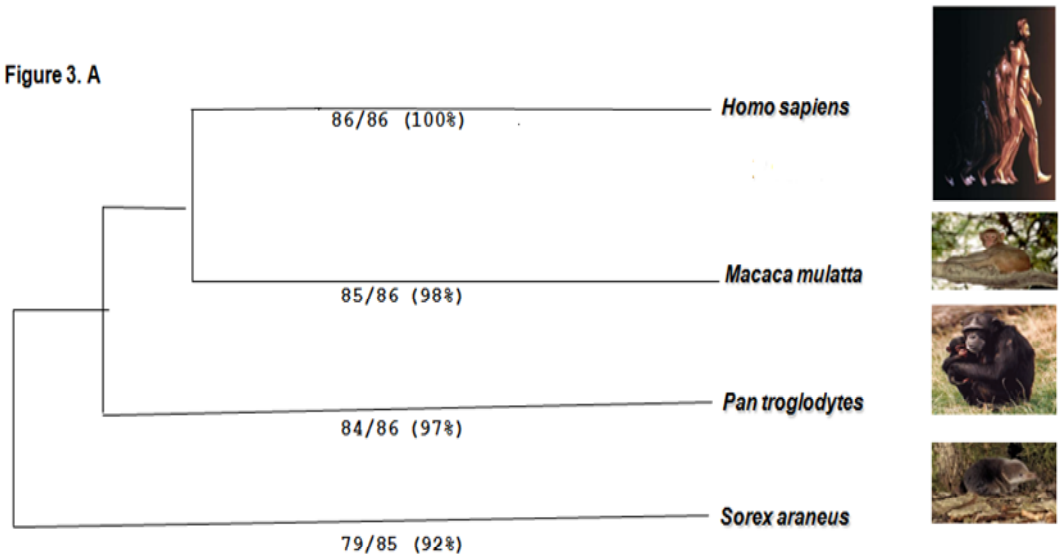
Third biogenesis pathway is like microRNA synthesis. Here we report the characterization of small RNA associated with human snoRNA, microRNA and piRNA by deep sequencing. We find a novel ncRNA (x-ncRNA) and more importantly it is having characteristics of three distinct family of ncRNAs which also have important functions in the maturation of other noncoding RNAs such as ribosomal RNAs. We have demonstrated that the bona fide x-ncRNA is processed to small ≅28 nt long RNA that stably associate with the process of microRNA and pi RNA.

Finally, we identify the expression pattern and its functions from the previously published report and the conclusion of its expression patterns in 2'O - methylation of rRNA, post-transcriptional regulation of gene expression by hybridizing to complementary regions of target mRNAs, possibly involved in transposon silencing (Nazar et al., 1980; Munholland and Nazar, 1987; Girard et al., 2006). Moreover its functional activity is in the subsets of microRNAs are expressed in most cell types (that is, tumor tissue) also spermatocytes and spermatids in testes. All these evidences are supporting the biogenesis pathway of x-ncRNA (Huttenhofer et al., 2001; Girard et al., 2006).

DISCUSSION

The microRNAs comparison of Homo sapiens helped to discover the new class of RNA. Our data hint that existence of novel x-ncRNA and bio genesis of x-ncRNA could be divided into three parts, based on piRNA, snoRNA and microRNA. In detail, the mature sequence of x-ncRNA is identified as a piRNA and it is reported as a germline-specific class of small RNAs that binds mammalian Piwi proteins. These reports elucidate that the dicer is involved to produce piRNAs even if not generated from the precursors (Houwing et al., 2007; Vagin et al., 2006). There are lots of evidence that piRNAs may arise from sequential processing of a longer and single strand (Kim, 2006). Since our results indicate that x-ncRNA precursor was processed and produced a piRNA, we are confirming that piRNA was processed from the double-stranded or hairpin precursor in Homo sapiens as like microRNA and snoRNA.

From snoRNA pathway, the precursor sequence of x-ncRNA was predicted as a snoRNA and it is reported as a small nucleolar RNA, C/D box 71, SNORD71, HBII-239 and its Accession Number is NR_003059 (sequence



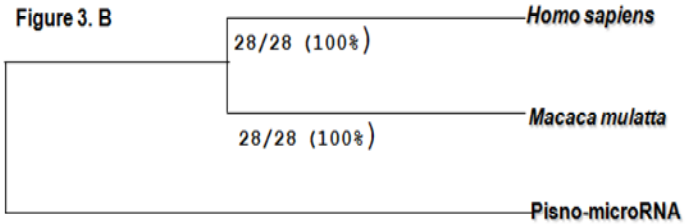


Figure 3. C

Homo sapiens	GTCACAATGCTGACACTCAAACTGCTGACA
Macaca mulatta	ATCACAATGCTGACACTCAAACTGCTGACA
Pan troglodytes	ATCACAATGCTGACACTCAAATTGCTGACA
Sorex araneus	ATCACAATGCTGAAACTCAGGTTGCTGACA

Figure 3. Evolutionary analysis of x-ncRNA. Phylogram derived from the genome blast analysis, by using pair wise x-ncRNA sequence relationship between the species. The percentages indicate the identities of the x-ncRNA sequence with the other species gene sequences. Pictures are the corresponding species of x-ncRNAs evolutionary tree.
A. Evolutionary tree constructed by using precursor gene sequence of x-ncRNA Evolutional relationship with *Macaca mulatta* 98%, *Pan troglodytes* 97%, *Sorex araneus* 92% identity.
B. Mature x-ncRNA gene evolutionary tree in different species. The analysis of mature gene sequences blasted with total genome elucidates that when *Homo sapiens* and *Macaca mulatta* share genes, the DNA sequences in these genes are 100% identical C. Mature x-ncRNA sequence of different species in which the presence of adenosine flanking in the 3' gene sequence.

length reported is 86 bp). It also has been accepted by HGNC, with ID number 32732. This suggests that x-ncRNA can have the functions as a snoRNA. The sno RNA HBII-239 is human orthologue of the mouse MBII-239 (Huttenhofer et al., 2001). HBII-239 is predicted to guide the 2'O-ribose methylation of 5.8S rRNA U14. Um14 is unique among all vertebrate to have rRNA ribose methylations and it takes place partially in the cytoplasm and nucleus, besides it is under methylated in tumor tissues (Munholland and Nazar,

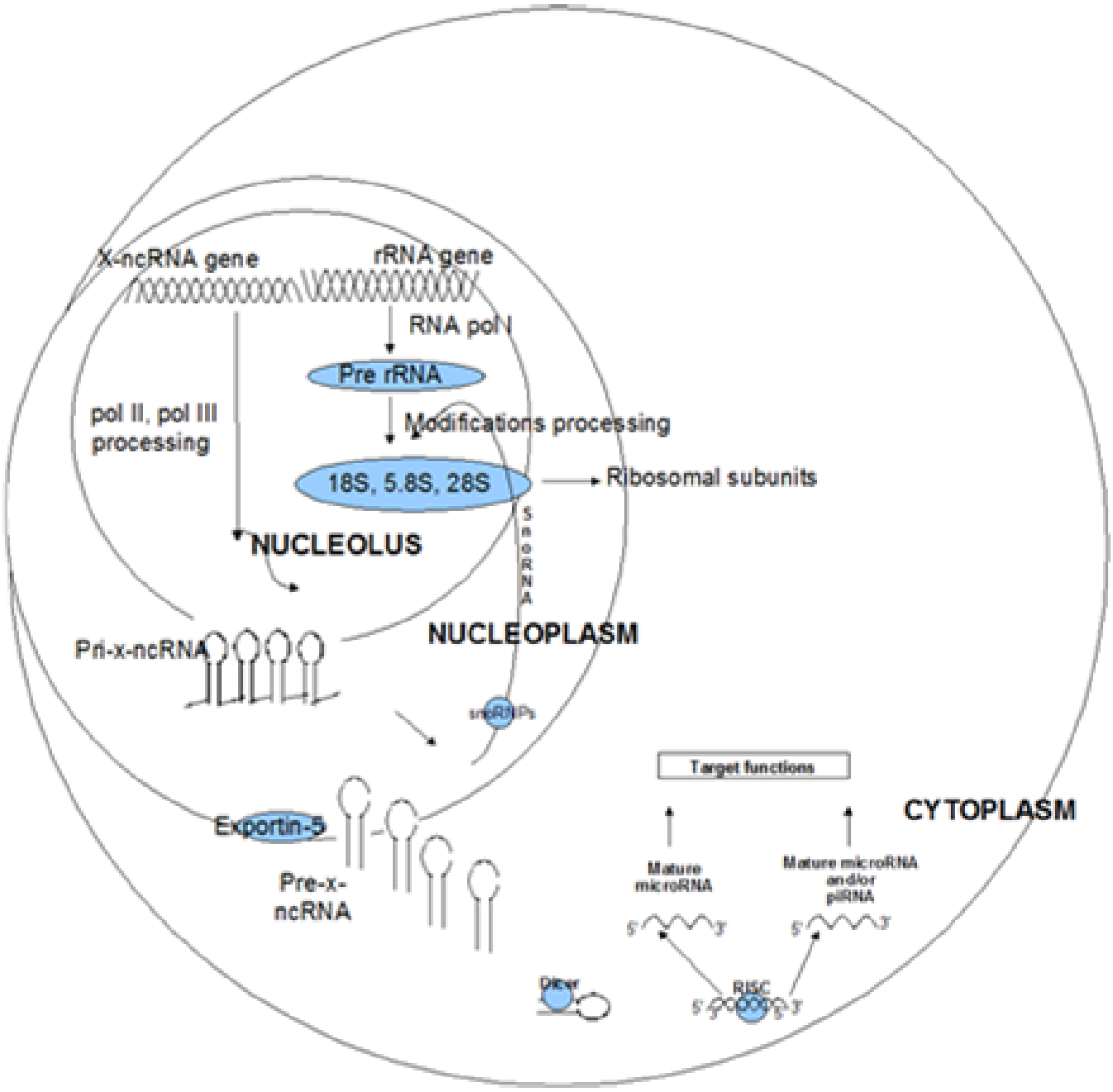


Figure 4. Human x-ncRNA bio genesis. This pathway is based on the discovery of x-ncRNA by using comparative analysis of piRNA, snoRNA and microRNA. In the first pathway x-ncRNA is processed as a snoRNA and involved in RNA modification processing. Discovered x-ncRNA especially involved in the guide 2'O-ribose methylation of 5.8S rRNA on residue U14. In the second pathway of x-ncRNA a precursor is processed as Pri-x-ncRNA and as Pre-x-ncRNA is exported into cytoplasm by exportin-5, furthermore dicer and RISC are involved to produce microRNA. In the third pathway of x-ncRNA precursor is processed as a piRNA through dicer or through a distinct pathway which is yet to be discovered.

We identified that the x-ncRNA precursor sequence is involved as a snoRNA in nucleus and its mature sequence as a piRNA and as a microRNA in the cytoplasm confirming that x-ncRNA could have functions in rRNA processing and also not related to rRNA processing (Figure 4) (Table 1). As regard as microRNA pathway, x-ncRNA precursor and mature sequence were described as a MIRN768 or hsa-mir768 which is an intronic micro-

Table 1. Properties of the human x-ncRNA.

Property	x-ncRNA	References
Length	Precursor, ≅104 nt Mature, ≅28nt.	Lestrade and Weber, 2006 Berezikov et al., 2006 Girard et al., 2006.
Processed RNA	small nucleolar RNA, C/D box 71, Snord 71, snoRNA HBII-239, MIRN768, microRNA 768, hsa-mir-768, intronic microRNA, piRNA.	Lestrade and Weber, 2006 Huttenhofer et al., 2001 Berezikov et al., 2006 Sung-chou et al., 2007 Girard et al., 2006.
Expression patterns	Subsets of microRNAs are expressed in most cell types (example, tumor tissue) also spermatocytes and spermatids in testes.	Nazar et al., 1980 Munholland and Nazar ., 1987 Girard et al., 2006.
Function	2'O - methylation of rRNA, post-transcriptional regulation of gene expression by hybridizing to complementary regions of target mRNAs, possibly involved in transposon silencing.	Huttenhofer et al., 2001 Girard et al., 2006.

RNA. The pre microRNA is involved as snoRNA precursor and the same precursor is further processed and exported into the cytoplasm to produce microRNA. This affirms that x-ncRNA precursor is processed to produce the pri and pre microRNA. mir-768 overlaps an annotated snoRNA, HBII-239. microRNA data base release version 10.1 was registered this as a microRNA and has-mir768 is mature and precursor miRNA (Registered in Sangers miRNA database), and its sequence length is 104 bp. In 2006, Berezikov reported this gene sequence and it has been approved as MIRN768 in HGNC and its ID number is 33142. Mature microRNA sequences, present on 16q22.2 chromosome and version 12.0 release shows that phylogenetic analysis in all vertebrates supports the snoRNA annotation, with poor conservation of the reported mature miRNA sequence, therefore this microRNA removed from the database. Even though the existence of these special characteristics seems to clarify it further.

Through these pathways described above we have predicted the x-ncRNA bio genesis pathway. x-ncRNA gene is transcribed as long primary transcripts (pri-microRNAs) by RNA polymerase II or polymerase III enzymes. This pri-microRNA cleaved into hairpin-shaped pre-microRNA by the nuclear RNase III drosha complex in one pathway. However, the same pri-microRNA can produce snoRNA by the snoRNPs. In the case of drosha intermediate process, the pre-microRNA is exported out of the nucleus by exportin-5 (Exp5) and it is subsequently cleaved by the cytoplasmic RNase III dicer into microRNA duplexes. The systemic analysis proven the same nucleotides processed as microRNA and piRNA by unknown molecular pathway since microRNA and/or microRNA mature sequence processed through dicer, which gave the hint that dicer may be involved in the production of piRNA.

The cytoplasmic process of HBII-239 suggests another possible pathway in which methylated mature sequence of HBII-239 snoRNA might be involved to produce the piRNA in the cytoplasm (Figure 4). This work also suggests the presence of the x-ncRNA, a novel RNA class with piRNA, snoRNA and microRNA characteristics. Besides, the presence of more x-ncRNA functions in the inter species and intra species should be discover for the better understanding of hidden molecular pathways in the x-ncRNA synthesis.

The novel class of x-ncRNA identification has extended the multi-faceted family of small interfering RNAs that includes microRNAs, siRNAs, piRNA etc. In summary, a novel class of ncRNA, x-ncRNA was identified in human. The identification of more functional x-ncRNA genes are important to identify genes linked to a particular disease and to understand post-transcriptional regulation of gene expression. Since x-ncRNA have the characteristics of three different families of ncRNAs, it promotes to know the function and its target regions. This unusual genomic cluster arising a lot to discuss. Several possible mechanisms have been proposed for piRNA biogenesis.

(I) Long-range dsRNA structures may be formed in primary transcripts, which may explain the strand bias of piRNAs.
(II) The antisense transcript may be expressed at such a low level that they escape detection.
(III) Primary transcripts may be digested by a ssRNA-directed unidentified enzyme.
(IV) Imai and colleagues proposed that piRNAs may be derived from RNA-DNA duplexes that are produced by the reverse transcriptase of retrotransposons (Aravin et al., 2006; Watanabe et al., 2006).

The recent paper by Ender et al. (A Human snoRNA with

MicroRNA-Like Functions) and by Wilusz et al. (3' End Processing of a Long Nuclear-Retained Noncoding RNA Yields a tRNA-like Cytoplasmic RNA) that shows that a given genomic region can be processed in two ways and give rise to two transcripts one of which is nuclear and the other cytoplasmic; in other words, there is precedent for multiply processed sequences with apparently different functionalities also supporting the existence of x-ncRNA as we discussed (Ender et al., 2008; Wilusz et al., 2008).

Our report proved that, the evolvement of a piRNA from the dsRNA like microRNA, (that is, same mature sequences of the transcript involved as a piRNA and microRNA) through dicer. It was previously shown that human Piwi proteins can interact with dicer (Sasaki et al., 2003) and our data determined the dicer and drosha are indeed involved in piRNA bio genesis. More over one strand of the duplex might be degraded, leaving a single-stranded mature miRNA and/or piRNA molecule, which combines with the members of the Argonaute protein family to form the RNA-induced silencing complex (Lin and Liu, 2009). Our predictions are also giving evidence for the presence of two families of piRNA that is, Dicer dependent piRNA and Dicer independent piRNA (Houwing et al., 2007).

To figure out these, we are looking forward for the direction of the genomic, proteomics and biochemical experiments that will support our model proposed, which could explains the function of x-ncRNA and its correlation with snoRNA, microRNA, piRNA along with its hidden secretes in small ncRNA.

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