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RESEARCH ARTICLE

Expression profiling and in silico characterization of bubaline Drosha in light of evolution*

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Abstract

Drosha is a class III ribonuclease and is a critical enzyme involved in miRNA biogenesis. The rationale behind the current study was to profile the tissue specific expression of bubaline Drosha and envisage its evolution in the light of other RNases of animal and prokaryotic origin. Relative quantification using qRT-PCR (TaqMan chemistry) revealed that its expression was the highest in kidney and heart and the lowest in brain. The clones of the overlapping partial cds of bubaline Drosha were custom sequenced. The sequences of Drosha and Dicer transcript variants of divergent origin were subjected to biocomputational analyses (using MEGA6 and Datamonkey server) which indicated that the bovidae, suidae, and marine mammals form different phylogenetic clusters. Wide range of variation among the divergent species indicated natural selection to confer specific functionality. To identify the selection in codons of the Drosha and Dicer the Ribonuclease domains of representative divergent sequences were analyzed using different models (SLAC, REL, FEL) of Datamonkey server. The salient findings were that certain codons of RNases have undergone purifying selection during evolution, the type III RNases have evolved independently of the RNase-L, -A and –H of animal origin from respective progenitors in the prokaryotic RNases and the domains of different RNases different markedly from each other as evidenced by the protein structure analyses. Finally, the divergent RNases were subjected functional classification and network analyses (Panther and String-db online tools) to identify their inter-relationship with respect to their functions. It was evident that the RNases of prokaryotic vis-a-vis animal origin are involved in specific networks according to the functional similarity. The overall findings indicate that the prokaryotic RNases have evolved with respect to the functional requirement and has acted as the progenitor of the more divergent animal RNases.

INTRODUCTION

Drosha, a class III RNase enzyme, is involved in miRNA biogenesis (Han et al., 2004) through constructing a microprocessor complex that recognizes the stem-loop structure of pri-miRNA, in cell nucleus (Bartel et al. 2004, Mejia et al., 2013). Drosha and Dicer, takes part in the stepwise processing of miRNAs, and these enzymes have key roles in miRNA-mediated gene regulation during cell differentiation, development etc (Gromak et al., 2013). The Indian water buffalo has not been extensively studied for the miRNA mediated gene repression and the causal enzymes involved in miRNA biogenesis. No report is available on tissue specific expression of the Drosha enzyme in buffalo. Besides, to date, the coding sequence of bubaline Drosha has not been cloned and biocomputationally studied in purview of evolution in animal kingdom and prokaryotes. RNases are very stable

enzyme and are indispensable for programmed lysis of excess mRNA in the cell. Various classes of RNases with specific targets have evolved during the process of evolution. In the present research work, the evolutionary perspective of bubaline Drosha with regard to Dicer has been studied with an aim to evolutionarily characterize Drosha and to decipher the inter-relationship among the RNases enzymes of animal and prokaryotic origin.

MATERIAL AND METHOD

Collection of samples :

Eight tissue samples of Indian water buffalo, namely, udder, ovary, liver, kidney, brain, heart, hoof base and testis have been collected (in RNA later solution) from healthy buffaloes immediately after slaughter at the slaughter house (Dera Bassi, Mohali, India).
Isolation of total RNA and cDNA synthesis:
The total RNA (1500 to 3000 ng/µL with absorbance ratio (260/280) between 1.9 and 2.1) was extracted from each homogenized tissues with 0.8 ml of Trizol (Invitrogen, USA) following precipitation with isopropanol (Sambrook and Russell, 2001) and then subjected to cDNA synthesis using first strand cDNA synthesis kit (Fermentas, USA) according to manufacturer's protocol.

Real time PCR:

TaqMan chemistry (Applied Biosystems, Life technologies) was used for relative quantification of the Drosha gene. The qPCR (40 cycles, three technical replicates) was carried out with 3 technical replicates in 30µl final volume containing 1X TaqMan master mix (TaqMan universal master-mix II with UNG, Life Technologies), primer-probe mix (900nM / primer, 200nM of probe) and template cDNA (2µl). Threshold cycle (Ct) values (baseline set at 0.2) were calculated using the SDS software v.2.3 (Applied Biosystems, Life technologies, USA). The relative quantification of the expression data (normalized with ß-actin and fold-change calibrated against brain tissue) was done using ddCt method (Livak and Schmittgen, 2001).

Polymerase chain reaction:

Eight pairs of primers (Table 1) targeting the overlapping partial fragments of Drosha coding sequence (cds) were designed from the available predicted Drosha cds (XM_005221630, XM_006067803) using Primer3 online tool (http://bioinfo.ut.ee/primer3-0.4.0/). PCR (Veriti, ABI) was done (in 25 µl) with 30 cycles of denaturation at 94ºC (45s), annealing (30s, Table 1 for primer-specific temperatures), extension at 72ºC (45s) and final extension was given at 72ºC (5 min).

Cloning and sequencing:

All the Drosha amplicons were cloned in pJET1.2/blunt cloning vector and transformed in DH5α cells. The positive clones were custom sequenced (after confirming by restriction digestion) from DNA Sequencing Facility, Department of Biochemistry, University of Delhi, South Campus, India.

Homology search:

The obtained partial nucleotide sequences of Drosha were processed and submitted to DDBJ (http://ddbj.nig.ac.jp/submission/). BLASTn (Altschul et al., 1990) search of the final coding sequence (cds) fetched the homologous, full-length Drosha cds (e-value < 10^-5) which were downloaded in FASTA format. The cds and amino acid sequences of other RNases like Dicer, RNase-L, -A, -H and prokaryotic RNases (Prokaryotic RNaseIII, RNaseH1, RNasePH-RraB, RNaseHIIPM1, RntRnr, RnpA, RnlA, Rra) were selected from the Nucleotide and Protein databases (of NCBI) for studying the evolutionary relationship between different RNAse enzymes. The RNase domains within the cds of Dicer, Drosha and all other RNAses were identified using SMART database (http://smart.embl-heidelberg.de/). A total of 60 nucleotide sequences of RiboC1 and RiboC2 domains of Dicer and Drosha as well as the respective RNase domains of other RNases (RNase-L, -H, -A of divergent animal species and prokaryotic RNases like prokaryotic RNaseIII, RNaseH1etc) were selected for further analyses and saved in FASTA format.

Multiple sequence alignment:

The domain specific nucleotide sequences of those 60 RNases were subjected to multiple sequence alignment using online tool MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/). The overall alignment window (not shown) was saved to depict the conserved regions of the RNases and ribonuclease domains.

Best evolutionary model selection:

The coding sequences of domains from various species were subjected to evolutionary analysis using MEGA 6 (Tamura et al., 2013). The best model (Kimura 2-parameter (K2)) was selected by determining the Akaike Information Criterion, corrected (AICc) values and was used for the further analyses of domains.

Phylogenetic tree construction:

MEGA6 software (Tamura et al., 2013) was used for construction of phylogenetic tree, estimation of evolutionary divergence, Fisher's exact test and codon based test for determining the selection pressure on the domains. The coding sequences were subjected to analysis for the phylogenetic tree construction using maximum likelihood method. The evolutionary divergence between coding sequences was estimated using the Kimura 2-parameter (K2) evolutionary model. The reliability of the branching of the tree was checked by 1000 bootstrap resampling.

Homogeneity of substitution patterns between sequences:

The disparity index (DI) estimates (by Monte Carlo test with 1000 replicates) for the target coding sequences of various species was estimated. The probability of rejecting the null hypothesis stating that the coding sequences have evolved with the same pattern of substitution based on the extent of differences in base composition biases between sequences (Kumar and Gadagkar, 2001). The heatmap for evolutionary divergence of the domains was generated using WGCNA package of R (version 3.2.1) program.

Determining the sites of positive or negative selection:

The specific codons that have experienced positive selection were determined using different statistical methods, namely, Single Likelihood Ancestor Counting (SLAC), Fixed Effects Likelihood (FEL), Internal branch FEL (IFEL) and Random Effects Likelihood (REL), of Datamonkey (www.datamonkey.org/) online server. A hierarchical testing amalgamated with nested LRT tests with AIC selection was done to test all of the 203 time-reversible models (http://www.datamonkey.org/help/models.php). Variation in rate of evolution along both branches and sites was adjusted by Branch-site REL tests (Pond et al., 2011) for episodic diversifying selection. This analysis enables to determine the lineages on which a subset of sites has evolved under positive selection, without requiring prior knowledge about which lineages are of interest.

Comparison of predicted structures of domains:

The predicted amino acid sequences (using Expasy Translation tool: http://web.expasy.org/translate/) of the domains (of DroshaRiboC1, DroshaRiboC2, DicerRiboC1, DicerRiboC2, RNase-A of buffalo; and prokaryotic RNase(RNase H of Desulfovibrio desulfuricans and E. Coli. RNase) were subjected to secondary structure prediction and ab initio tertiary structure prediction using online tools Psipred v3.0 (http://bioinf.cs.ucl.ac.uk/psipred/) and RaptorX (http://raptorx.uchicago.edu/) (Lovell et al., 2003), respectively. The 3D protein structure obtained from RaptorX was subjected to structure validation using WhatIf online tool (http://swift.cmbi.ru.nl/whatif/) and Ramachandran's plot analysis was done for validation of the predicted protein structure using RAMPAGE online tool (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

Network analysis and gene ontology:

The UniProtKB gene-symbols of the genes under study (E1BGY0, Q6TUI4, A5H027, Q2TBT5, Q2NKV1, P08904, Q3ZC23, Q7M330 for taurine Drosha, Dicer1, Rnase L, Rnase H, Rnase A1, Rnase A k6, RNase K , Rnase T2, respectively; and P0A7Y0, P21499, I1RGU1 , P0AF90, P0A7Y8 , P52129, P0A8R0, P30014 for rnc, rnr, FGSG_02968, rraB, rnpA, rnlA, rraA and rnt, respectively of E. coli) were obtained from http://www.uniprot.org/. The gene-symbols were subjected to network analysis (separately for each group) using String-db (http://string-db.org/) after selecting cow and Escherichia coli K-12 MG1655 as the organisms. The gene Ids were also subjected to Panther analysis system (http://pantherdb.org/) for gene ontology classification for biological functions (separately for cow and E. coli).

RESULTS AND DISCUSSION

Cloning and sequencing:

The RE-digested recombinant vectors (containing the amplicons of the overlapping primers) showed single specific bands of the respective inserts (Table 1) on submarine gel electrophoresis when run in 1-2% agarose gel prepared in 1X TAE buffer. These fragments were cloned and custom sequenced for obtaining the full length coding sequence of Drosha (Figure 1; DDBJ Getentry accession number: LC077859). Earlier, Zhang and Cho cloned and characterized 3 RNases genes of Zebrafish to understand their evolutionary origin (Cho and Zhang, 2007).

Expression profiling using Real time PCR:

Tissue specific expression of bubaline Drosha was profiled after calibrating the expression with respect to that of brain. Drosha enzyme exhibited highly differential pattern of expression across the tissues being studied (Figure 2) with the highest expression in kidney followed by heart tissue. Quantifying the expression of protein or enzyme across the different tissues is an important initial step to investigate its functions as well as to provide a reference for comparing the expression in different physiological conditions. The altered expression of Drosha and Dicer was compared in malignant breast-tissues with normal tissues in human to find out markers for depraved miRNA expression during tumorogenesis (Avery-Kiejda et al., 2014).

Sequence analysis of Ribonuclease domains and RNases:

The analysis was performed on the coding sequences of RiboC domains of Dicer and Drosha and the RNase domains of RNase-A, -L, -H of animals and various RNases of prokaryotic origin. Coding sequences (of open reading frame) were preferred over the translated amino acid sequences for the evolutionary analyses as the coding sequences are longer than the amino acid sequences while the sequence alignment of amino acid merely revealed any column with identical residue. The best model for further evolutionary analyses was Kimura 2-parameter (K80) (Kimura, 1980) with the lowest BIC (2945.59) value out of 24 evolutionary models examined. In a study on bubaline Dicer I cds, Singh et al. (2015) from this lab analyzed the evolutionary perspective with respect to divergent Dicer sequences from animal kingdom.

Sequence alignment, phylogeny construction and evolutionary divergence of the domains:

Multiple sequence alignment of the divergent open reading frames (ORFs) of the domains (identified using SMART analysis service (http://smart.embl-heidelberg.de/)) belonging to different types of RNases revealed the conserved regions of domains among the different species. These domains are positionaly conserved in different species with some variation between some species. The phylogenetic tree (Figure 3) clearly revealed distinct clustering of Dicer-RiboC1, Dicer-RiboC2, Drosha-RiboC1 (except for fruit-fly, threadworm), Drosha-RiboC2, RNase-H, RNase-A (except for that of fruit fly), RNase-L (except for seal and monkey). Drosha RiboC1 of fruit fly and threadworm forms a very distant branch which suggests its distant relationship with the other domains, but the closest with the RNase-L domain of animals. The domains of Drosha RiboC1, RNase-A and RNase-L exhibited slight diffused type clustering with some of the taxa clustering away from the main cluster of that particular domains, while, interestingly, the prokaryotic RNases revealed the maximum amount of dispersive nature. Different prokaryotic RNases revealed closeness to different certain RNase domain-clusters of animal origin, viz. Prokaryotic RNase III with Drosha (RiboC1 and 2) and Dicer (RiboC2) RNase III domains, similarly, E coli RnlA with Dicer RiboC1 and RNase-H and –A; several prokaryotic RNase (Rnr, RNasePM1, RraB, Rnt) with animal-RNase-L. Murphy and coworkers (2008) studied the evolutionary relationships of four major proteins (RISC RNA-binding proteins, Dicer, Argonaute and Exportin-5) involved in miRNA pathway and showed that they are derived from multiprotein families that seems to be a common trend in the evolution of miRNA pathway.

The input sequences were grouped into the source genes like RiboC1 and 2 domains of Dicer and Drosha, RNase-A, -L, -H of animals, and different prokaryotic RNases like, RNase-III, -H1, PH-RraB, HII PM1, -RntRnr, RnpA, RnlA and Rra (unrooted tree is boxed in Figure 3). The evolutionary divergence heat map represented the average number of base substitutions per site for all sequence pairs between groups to measure the departure of sequences between two or more groups of species with respect to certain parameter (Figure 4A). The heatmap clearly depicted that the eukaryotic RNases are the evolutionary procreation of specific prokaryotic RNases, as indicated in the phylogenetic tree. It is for sure that the rate of evolution can not be the same for all the RNase-

linages, starting from the prokaryotes. In present day situation, the eukaryotic RNase-L has diverged considerably from Dicer (RiboC1 domain), similarly RNase-H from all Dicers and RNase-A. Report suggests that Dcr2 and Ago2 are the fastest evolving genes in D. melanogaster (Obbard et al., 2006). Mukherjee et al. (2012) showed that the Dicer genes duplicated and diversified independently in early animal and plant evolution by conducting the evolutionary analysis of ribonucleaseIII enzyme Dicer in eukaryotes.

Homogeneity of substitution patterns between sequences:

Disparity index measures the observed difference in evolutionary patterns for a pair of sequences through the probability of rejecting the null hypothesis (H_0) of same pattern of substitution among different lineages. The H_0 was rejected ($P<0.05$) for most of eukaryotes but some RNases in case of prokaryotes (Figure 4B). Some domains of the RNases have shown significant disparity during evolution indicating substitution pattern varied among them. This indicates that the branching pattern obtained in the phylogenetic tree could be misleading, since the assumption of homogeneity of substitution pattern has not been met.

To address this question, the coding sequence data of the ribonuclease domains and the RNases have been subjected to "branch-site REL analysis" (BSR) (Pond et al., 2011; Pond and Frost, 2005) in Datamonkey online server to analyze the episodic diversifying selection of divergent homologous coding sequences. In total 12 branches (or nodes) could be detected ($p<0.05$) to undergo episodic diversifying selection (Figure 5). The colors of the branches of the tree signify strength of selection: blue corresponds to purifying selection ($\omega = 0$), black or grey to neutral or nearly neutral ($\omega = 1$) and red color corresponding to diversifying (or positive) selection ($\omega > 5$). On the other hand, the width of the branch corresponds to the proportion of sites undergoing episodic diversifying selection. This suggests that these sequences have been evolved under heterogeneity of substitution pattern. Mukherjee et al. (2012) also used the branch-sites analyses in a study conducted on Dicer2 enzyme of flies and concluded that the helicase and PAZ domains of Dicer have experienced positive selection.

Estimation of selection pressure for various codons:

The test statistic "dN-dS" is an indicator of the selection pressure due to positive, negative or neutral selection, being operative on that codon. The positive and negatively selected codons were different for SLAC (28 and 7 sites, respectively), REL method (15 and 3, respectively) and FEL method (20 and 7, respectively) in the aligned cds. Thus in fine, it can be concluded that the number of positively selected sites is more than the number of negatively selected sites. Besides, rest of the aligned codons is selectively neutral. Hence, specific codons have experienced purifying selection during evolution to dispose the other types of variations which were possibly unfit to survive the selection pressure.

The results clearly indicated that almost all the positively selected sites are positioned in the highly variable regions of domains and RNases. This indicates that the various domains and RNases which are persisting in the species under study have got specific role in the evolution of the organism. The stringency of structural and functional constraints imposed on the protein structure determines the rate of amino acid substitution. The proteins with very stringent structural or functional requirements are subjected to strong negative selection pressure which limits the number of changes in the gene product (Kimura; 1983, Nei; 1987, Li; 1997).

Graur (1985) analyzed mammalian genes and proposed that the functional constraints have only a minor effect whereas the protein-composition is the critical feature that determines the rate of evolution of proteins. On the contrary, Tourasse and Li (2000) concluded that functional requirement is the cornerstone that governs the rate of protein evolution and this is weakly affected by amino acid composition. As a consequence, functionally critical regions, viz. domains, motifs, catalytic sites or binding sites of a given protein, are more conserved than the rest of the molecule.

Comparative analysis of protein structure prediction:

Protein structure prediction analysis was done for eleven different sequences representing various domains and RNases. The 3D protein structure analysis showed pleated sheets, α- helix and loop structures (Figure 6, Table 2). Secondary structure prediction of the sequences revealed the beta sheets are present only in five sequences namely RiboC2 domain of Drosha, RNase of E.coli, RNase-A of Buffalo, RNaseH1 and RNaseH2 of prokaryotes. The number of coils and helixes varies among different types of RNases and also between different species. These modifications emerge from the selection pressure over the different types of RNases. The evolution of the types of RNases must have taken place independently in such a manner that the type III RNases (Dicer and Drosha) have evolved independently of the RNase-A, -L and –H of animal origin. However, the prokaryotic RNases of different

types have acted as the progenitor of these different types of the RNases. Our finding is supported by a similar study has been conducted to understand the acquirement of function by two RNases of primates during evolution. Positive Darwinism selection contribute to diversification of these genes (Zhang et al., 1999). In an another study conducted on eubacterial RNase P, secondary structure has been elucidated by phylogenetic comparative approach (Brown et al.,1991).

Gene Networking and Functional Classification:

The gene network of the cattle (representative of animal RNases) clearly depicted that Drosha, Pasha and Dicer (along with Ago proteins) being a component of miRNA biogenesis pathway, form a cluster, while rest of the RNases are not a part of any network (Figure 7A). It suggests that these RNases (-L, -K, -H, -E) are distinct in their role and has evolved independent to each other with specific functions. Similarly, the prokaryotic RNases reveal inter-related association for most of the RNases. The rnr (encodes RNase R which is involved in maturation of rRNA, mRNA degradation during stationary phase, polyadenylated mRNAs degradation and tmRNA-mediated degradation of non-stop mRNAs), rnd (an exonuclease RNase D ,is involved in 3' ribonucleolytic processing of precursor tRNA) and rph (RNase PH acts in processing of the tRNA 3' terminus) are inter-related due to similar activity. Interestingly, the prokaryotic rnc (RNase III: acts in processing of ribosomal RNA (rRNA) and phage mRNA) has no association with other prominent E. coli RNases (rnr, rnp etc) except for recO (in in RecA-mediated replication recovery), rhlB (helicase component of degradosome; in association with srmB, PNPase, Rnase E) all of which exhibit functions in similar direction (Figure 7B). So comparative analysis of these two network indicates that the RNases in the prokaryotes were already assigned specific work and they were evolved possibly through gene duplication and then target specific diversification. While the eukaryotic RNases are the advanced, refined and more heavy-duty progenies of these prokaryotic RNases. The gene ontology study (Figure 7C and 7D) itself explains that the functional features of the prokaryotic RNases have diverged and become more robust over time to include more functions in the eukaryotic complex system.

Table 1: Detail of the PCR-primer-pairs used as gene specific primers for amplifying the overlapping partial fragments of the bubaline Drosha cDNA

Primer	Primer Sequence (5'-3')		Size[$]	Ta*	Accession No.
	Forward	Reverse			
2Dic2	tacgtcacgatgcaaggcag	gggctgttctggaagctact	585	52	LC066938.1
Gap1	cagcagtagccctcacttca	ctcgtgatccgacctgtagc	249	52	
Jn1-1	aacgtaaaaaggcccatcct	ctgcgtatttctgccactca	665	52	LC065664.1
2Dic5	ggtccccagaaaggctaaag	agagcaggtgctgtcctcat	824	58	LC065663.1
2Dic7	ggtggagctgagtagccaag	ttggctcttgaagctggagt	758	56	LC061127.1
2Dic8	gcttcagtgggaggaacttg	gaactccaaccgttcgttgt	745	58	LC054298.1
2Dic9	tccagcttcaagagccaaat	cttcattcagcgtctccaca	971	56	LC054299.1
4Dic3	catggatcaggtgggagatt	agtttgtgggaggtgagactg	472	56	LC061128.1

$ Size of the insert (bp)

* Ta: Annealing temperature (°C)

Table 2: Secondary and tertiary structure prediction vis-a-vis Ramachandran plot for the selected sequences of RNase and domains

RNase domain & species	#H-S-C	P-Value	# Amino acids	Number of Residues in the regions		
				Favoured*	Allowed**	Outlier
Bubaline DroshaRiboC1	6-0-7	6.91E-05	119	111(94.9%)	6(5.1%)	0
Bubaline DroshaRiboC2	7-1-9	2.40E-06	133	127(96.9%)	1(0.8%)	3(2.3%)
Bubaline DicerRiboC1	4-0-5	1.46E-05	81	76(96.2%)	0	3(3.8%)
Bubaline DicerRiboC2	8-0-9	4.93E-07	165	160(98.2%)	2(1.2%)	1(0.6%)

E coli RNase	4-10-15	2.18E-11	212	205(97.6%)	3(1.4%)	2(1%)
Bubaline RNase-A	4-6-11	1.73E-08	146	123(93.9%)	7(5.3%)	1(0.8%)
Bubaline RNase-L	9-0-10	8.39E-06	117	692(96%)	27(3.7%)	2(0.3%)
Green monkey RNase-L	8-0-9	1.77E-06	134	127(96.2%)	3(2.3%)	2(1.5%)
Bdellovibrio bacteriovorus RNaseHII	5-4-9	1.13E-08	180	170(95.5%)	4(2.2%)	4(2.2%)
Desulfovibrio desulfuricans RNase-H1	4-5-9	5.92E-08	135	129(97%)	4(3%)	0

#H-S-C: Number of helix, coils and sheets vis-a-vis correpsonding P-value

* Against expected number of residues in favoured region 98 for all the sequences as revealed by Ramachandran's plot analysis

** Against expected number of residues in allowed region 2 for all the sequences as revealed by Ramachandran's plot analysis

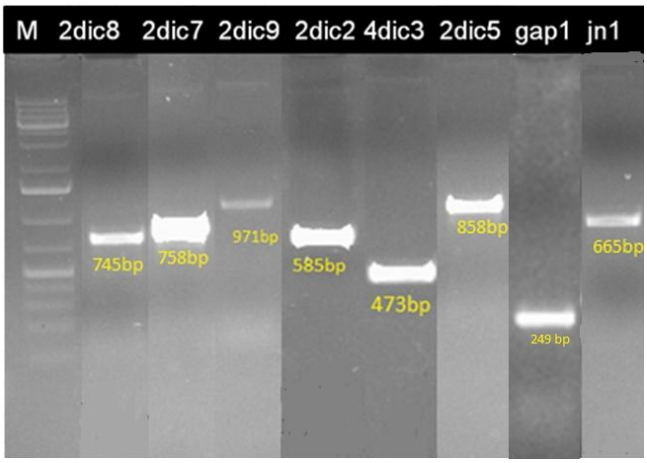

Figure 1: Plasmid PCR for the confirmation of clones of primers 2Dic7,2Dic8,2Dic9 2Dic2,4Dic3,2Dic5,Gap-1 and Jn-1

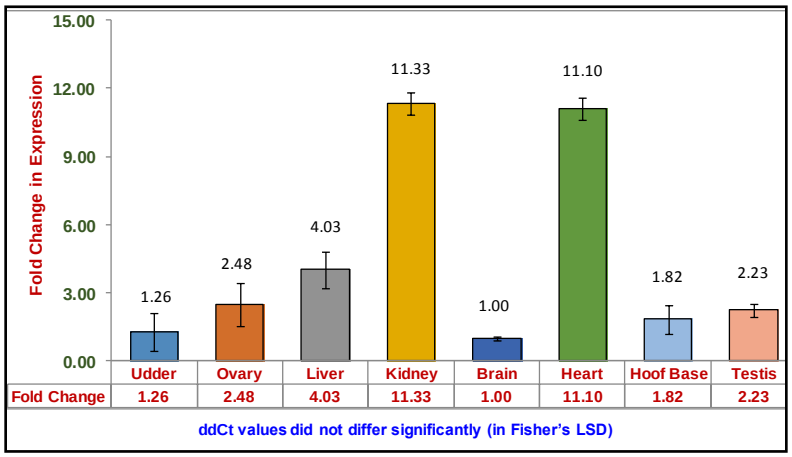

Figure 2: Relative expression profiling of Drosha gene in terms of fold change with respect to brain, in eight bubaline tissues

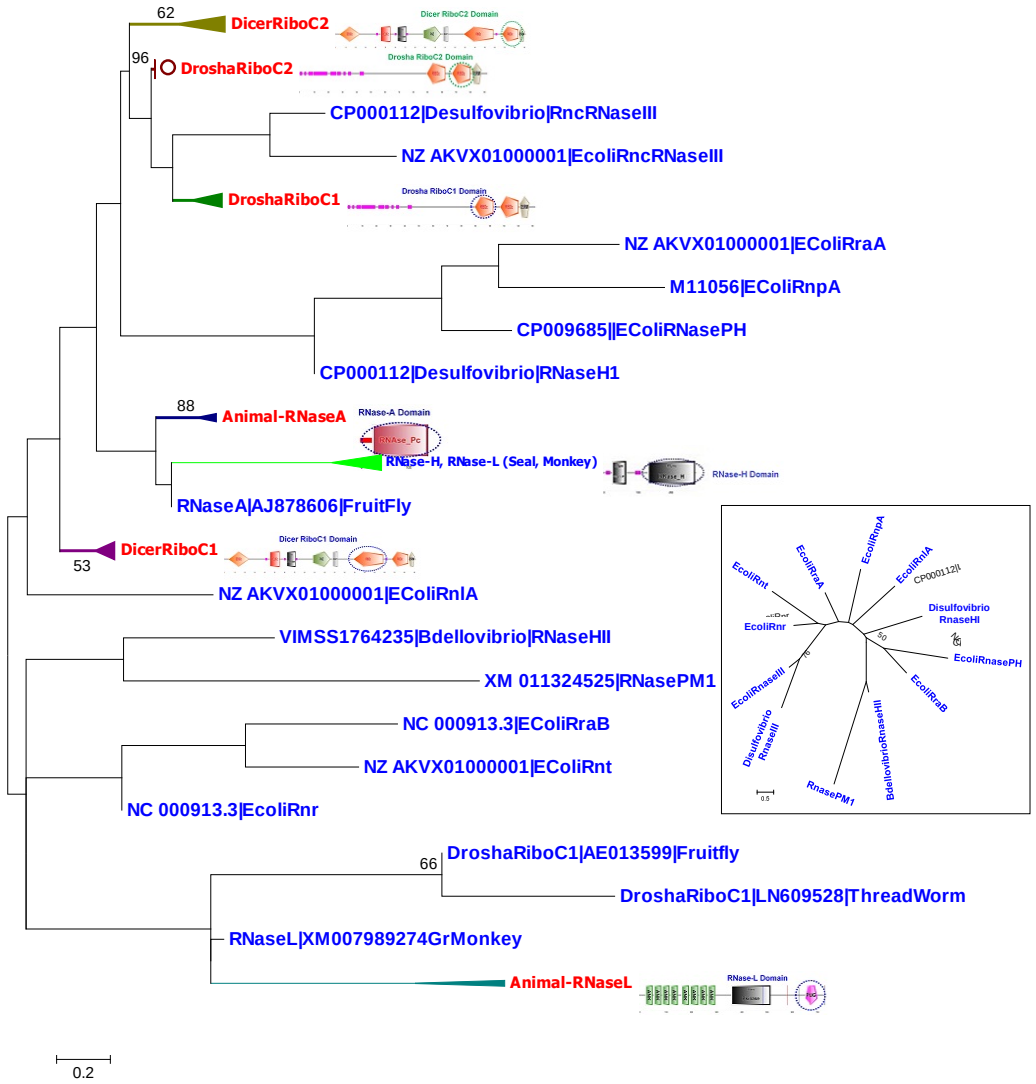

Figure 3: Phylogenetic tree constructed from the open reading frames (ORFs) of the divergent RNase domains belonging to animal and prokaryotic species, using maximum likelihood method with 1000 bootstrap resampling. The unrooted tree within the box depicts the phylogeny of the prokaryotic RNases

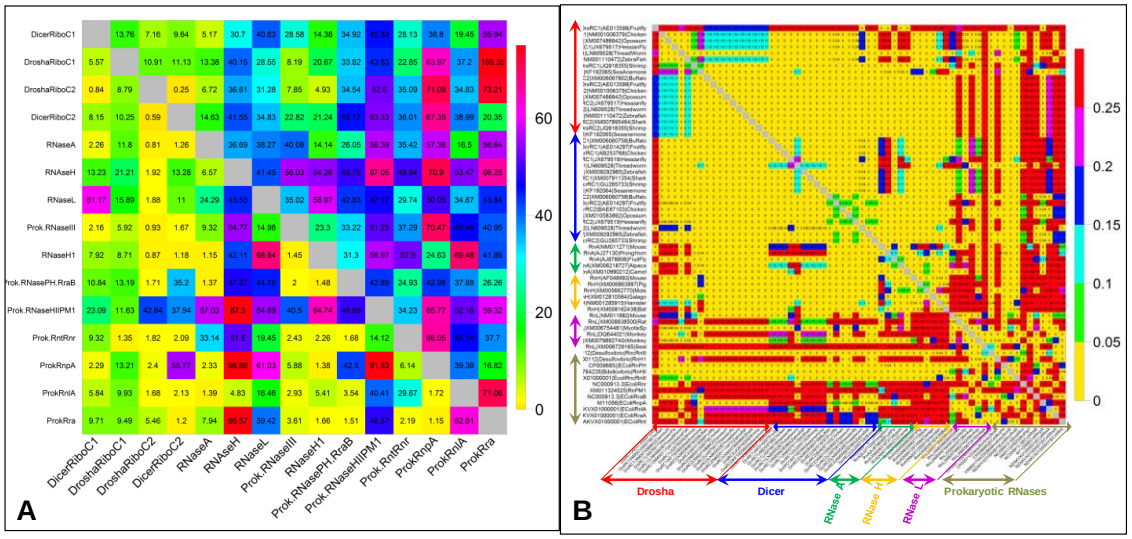

Figure 4: Relative distances between different RNases belonging to animal and prokaryotic origins (below diagonal) and the respective standard errors (above diagonal) depicted by evolutionary divergence heat map (4A), and Probability of rejecting the null hypothesis of Disparity Index indicated by yellow color ($P<0.05$) (4B)

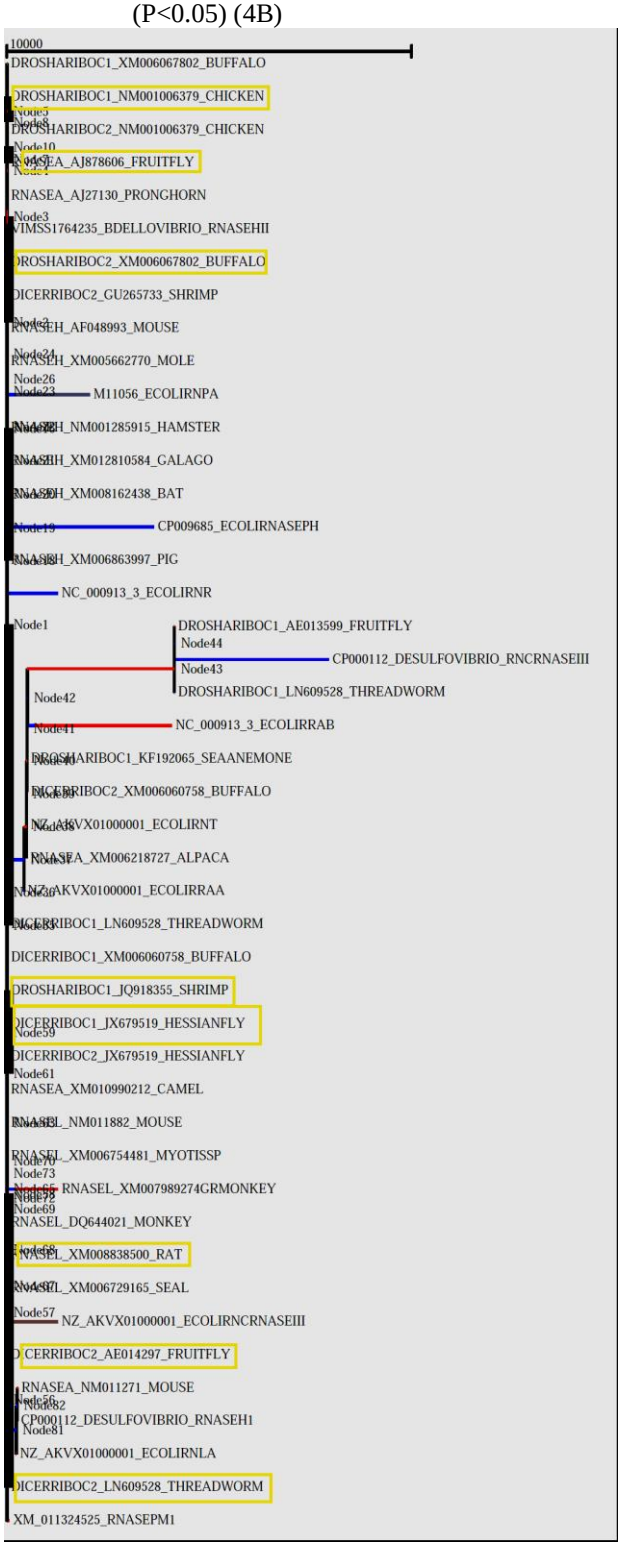

Figure 5: Branch-site REL analysis of all RNase domains to identify the sequences which have undergone episodic diversifying selection. The yellow colored rectangle indicates the sequence undergoing episodic diversifying selection

Figure 6: Tertiary structure of RiboC domains of bubaline Dicer and Drosha, RNase-A, -H, -L and prokaryotic RNase predicted ab initio using the online software RaptorX

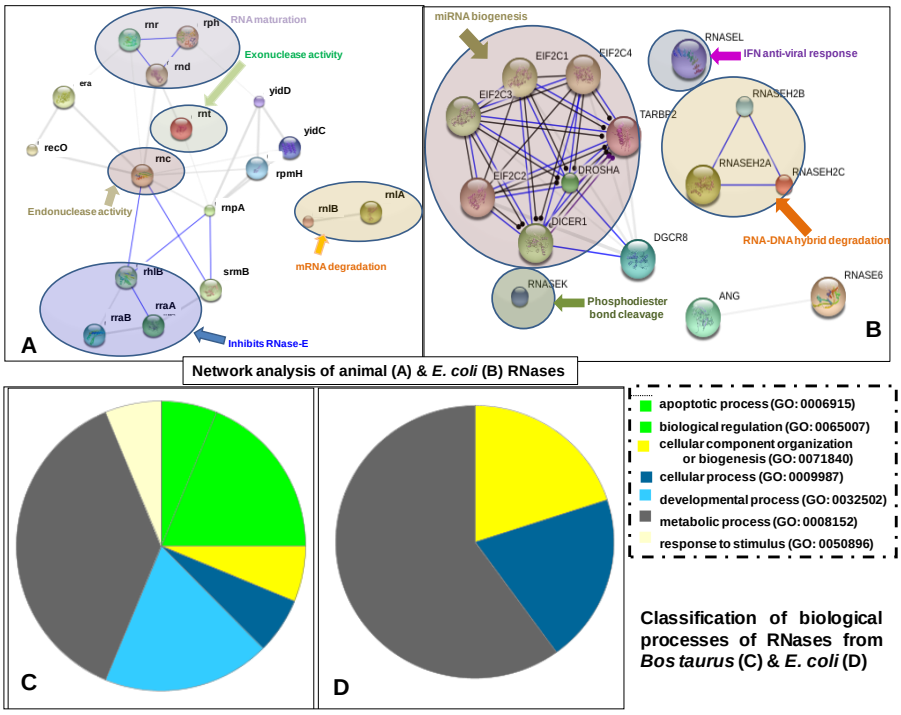

Figure 7: Functional analyses of the RNases from prokaryotic (source E. coli) and animal (source Bos taurus) origin. The molecular inter-relationship in terms of gene-networks (Figure 7A and B) of the enzymes has been depicted. The biological processes associated with the genes under study have been functionally classified for each group (Figure 7C and D) using Panther classification system of gene ontology

CONCLUSION

The expression profiling of Drosha in different tissues will enable researchers to hypothesize on differential miRNA expression and thereby gene knockdown in different tissues. The Drosha and Dicer genes have evolved independently from the other RNases genes in animal kingdom, from the specific precursor prokaryotic RNases through codon specific positive and negative selection.

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