Molecular Cell, Volume *28*

Supplemental Data

Mammalian Mirtron Genes

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The figures are included within the corresponding PDF file.

Figure S1. Mammalian Computational Mirtron Candidates with Saddle-Like Conservation Pattern and Single-Arm 2nd Structure in at Least Some Primate and Nonprimate Species

Front page summarizes information on the locus, orthologs that passed minimum mirtronic-hairpin criteria, and a representative secondary structure of a primate ortholog. Additional pages present detailed information on the genomic region, presence of spliced ESTs, and saddle shaped conservation profile, as obtained from the http://genome.ucsc.edu.

Figure S2. Conserved Hairpin Structures and Sequence of the Conserved Mammalian Mirtron, *mir-1225*

Secondary structures generated by RNAshapes are shown in bracket notation, and multiple alignment of these orthologous introns was generated by ClustalW.

Figure S3. Small RNA Libraries Used for Mirtron Discovery

Figure S4. List of Officially Recognized Mammalian Mirtrons and the Extent of Their Cloning Evidence

Library codes are described in Fig. S3.

Figure S5. Sequence and Secondary Structures of Orthologous Primate Introns, Whose Cloned Small RNAs Were Found in Only One Species

It is evident that most of these are clearly conserved in human/rhesus/chimp, whereas certain orthologues appear less compelling.

Figure S6. List of Candidate Mammalian Mirtrons with Atypical Hairpin Structures or Minimal Cloning Evidence

Figure S7. Correlation between Number of Mirtron Small RNA Reads and Number of Human ESTs

EST numbers were calculated in two ways: ESTs that include the mirtron region, and ESTs in the entire gene (which includes a much larger number of clones). Several abundantly cloned mirtrons have many ESTs, but there are also abundantly-expressed mRNAs that do not give rise to many cloned small RNAs and some abundant mirtron RNAs with comparatively few host ESTs. The lack of a strict correlation argues against mirtron-derived small RNAs being mere degradation products that are cloned in proportion to the expression level of the host mRNA.

Figure S8. Minimum Free Energies (MFEs) of 5 nt Terminal Duplexes of Mirtronic miRNAs

We calculated the relative free energy of the ends of the cloned miRNA/miRNA* duplexes and asked whether that was correlated with the predominantly cloned arm. In

most cases, as predicted by thermodynamic rules, the arm whose 5' end resided in a higher free energy duplex was preferentially cloned. The major exception was *mir-1226*, whose 3' arm was dominantly cloned, even though energy rules predicted its 5' arm as dominant.

Figure S9. MiRbase 10.0 *Drosophila*, Human, and Mouse Mature miRNAs Annotated with a 5' G Nucleotide

Data were extracted from http://microrna.sanger.ac.uk/sequences/index.shtml, Release 10.0: August 2007.

Figure S10. Cloned Human miRNA/miRNA* Duplexes that Do Not Have 2nt-3' Overhangs in Their Pre-miRNA

Although it is uncertain whether the miRNA* species of all of these genes have been accurately annotated, many of these are abundantly cloned miRNA/miRNA* pairs. The existence of atypical end structures suggests that there is not an absolute rule for pre-miRNAs to have precise 2-nt 3' overhangs.