THE FAMILY OF SMALL NON-CODING RNAs

The new regulators of gene expression and genome integrity
THE NEW WORLD OF RNA

Researchers are discovering that small RNA molecules play a surprising variety of key roles in cells:

- Inhibition of translation of mRNA into protein
- Degradation of messenger RNAs
- Genome defenders (Virus–Transposons)
- Chromatin organization
THE FAMILY OF NON–CODING RNAs

They collectively function as sequence-specific guides to silence or regulate genes, transposons, and viruses and to modify chromatin and genome structure.

The small non-coding RNA group

- ~21 nt
  - siRNAs (short interfering RNA)
  - miRNAs (micro RNA)
  - piRNAs (PIWI-associated)

Small non-coding RNAs

- 20-35 nt (siRNA, piRNA, miRNA)

Long non-coding RNAs

- 500 nt (Telomerase RNA)
- 17 kb (Human Xist)
- 108 kb (mouse Air RNAs)
Nature abhors double-stranded RNA. When confronted with double-stranded RNA (dsRNA), eukaryotic cells respond eliminating their own mRNAs that share sequences with the double strand.

Sources of siRNA are dsRNA that are typically hundreds of base pair long (viral- or transposon-derived replication intermediates).
RNAi was first discovered in *C. elegans* as a response to exogenous dsRNA

- RNAi could be provoked by:
  - injection of dsRNA
  - feeding of dsRNA
  - exposure to only a few molecules of dsRNA triggers systemic gene silencing and in its F1 progeny.

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Biogenesis of Effectors siRNAs

dsRNA-specific RNase III (Dicer)

dsRNA → Dicer, ATP → Guide strand

3'-OH → 3'-OH → 5'-PO₄

siRNA

Passenger strand

RISC

Ago

mRNA halves

mRNA

Guide strand → RNA-INDUCED SILENCING COMPLEX
RNAi can be elicited exogenously, by dsRNA supplied from outside the cell, or endogenously, from transcription of coding or noncoding genomic sequences.
THE ARGONAUTE FAMILY
Prime components of small RNA effector complexes
Defined by 2 main domains: PAZ (Piwi-Argonaute-Zwile) and PIWI (P-element Induced Wimpy Testis)

- **PIWI** domain is a RNAse-H like moiety (Slicer)
- **PAZ** domain identify the 3’OH end of small RNA

Piwi genes were originally identified as encoding regulatory proteins responsible for maintaining the undifferentiated state and division rates of germinal stem cells in *Drosophila*. Highly conserved and present in plants, animals and prokaryotes.
The ARGONAUTE family can be divided into 2 subfamilies:

- **Argonaute-like** (*Arabidopsis*) Expressed ubiquitously and associated in silencing complex of siRNA and miRNA

- **Piwi-like** (*Drosophila*) Expression restricted to Germinal and Stem cells (human and mouse orthologs are dubbed Hiwi and Miwi)

Piwi proteins associate with 27-31 nt small RNAs which match to genomic repeats and transposable DNA elements
PIWI–ASSOCIATED SMALL RNAs – piRNAs –

- A distinct class of ~ 30 nt RNAs associated with Piwi-like proteins
- Dicer independent biogenesis
- Described in flies, fish and mammals
- Essentials in maintaining germline and stem cell DNA integrity
- piRNAs assures the primary control of chromosome structure, chromatin organization, gene transcription, RNA stability and RNA translation

http://pirnabank.ibab.ac.in/  piRNAs registered: human ~23.439, mouse ~39.986, rat ~38.549
THE MICRORNA (miRNA) FAMILY
-- a novel class of small non-coding RNA molecules --

![Graph showing PubMed citations for miRNA over years 2001 to 2008. The bars indicate a significant increase in citations from 2005 onwards.](chart.png)
The First microRNAs (Small Temporal RNAs)
-- The role of heterochronic genes in *C. elegans* development --

Antisense incomplete complementarity to multiple sites in the 3’ UTR of mRNAs
Heterochronic genes do not encode proteins but small RNA transcripts of 21nt and ~70nt.

- Mutations in heterochronic genes cause temporal transformations in cell fates in which stage-specific events are omitted or reiterated.

  \textit{lin-4} \hspace{1cm} (Horvitz et al. 1980; Lee et al. 1993)

  \textit{let-7} \hspace{1cm} (Reinhard et al, 2000)

The first microRNA
Small Temporal RNAs
-- Conservation of let-7 sequence and expression in humans --

The *let-7* RNA is conserved across all the bilaterian phylogeny of metazoans
(Pasquinelli et al., 2000)

BLASTN searches reveal one gene for *D. melanogaster* or *C. elegans*, and three segments from the human genome sequence on chromosomes 9, 11 and 22 bearing exact sequence matches.
To date there are more than 9,500 miRNA genes reported with >700 correspond to the human genome (http://microrna.sanger.ac.uk/sequences/index.shtml)

miRNAs induce degradation or translational inhibition of target mRNAs after binding to the untranslated 3’UTRs.

miRNAs are estimated to comprise ~2% of animal genes and regulate more than 20% of genes.
miRNAs TRANSCRIPTION AND MATURATION

Small RNAs
- siRNA
- miRNA
- piRNA

Diagram:
- miRNA gene
- RNA Pol II
- pri-miRNA
- Drosa-DGCR8 complex
- pre-miRNA
- Exportin 5
- Dicer
- miRNA duplex
- RISC
- mature miRNA incorporated into RISC
- miRNA* strand degradation
- mRNA targeting
SHARED BIOSYNTHETIC PATHWAYS OF siRNAs AND miRNAs

Small RNAs

siRNA  
miRNA  
piRNA

Nucleus

Cytoplasm

Drosha

Exportin 5

miRNA

Gene

Pri-miRNA

Pre-miRNA

Cytoplasm

RISC assembly

RISC

Ribosome

mRNA cleavage

Translational repression

Asymmetric RISC assembly

Some miRNA

Dicer

Unwind

siRNA
duplex

miRNA:
duplex

dsRNA
Approx. 30% of miRNA genes are in intergenic regions

Approx. 70% of miRNA genes are located in defined transcription units

~25%  ~75%  ~1%
**GENOMIC LOCALIZATION OF miRNAs GENES**

**Canonical miRNA genes**

- **a** Non-coding TU with intronic miRNA
  - DLEU2

- **b** Non-coding TU with exonic miRNA
  - BIC

- **c** Coding TU with intronic miRNA
  - MCM7

- **d** Coding TU with exonic miRNA
  - CAONG8

**Non-canonical miRNA genes (mirtrons)**

- pre-mRNA
  - Spliceosome
    - Splicing
    - Mature miRNA
      - Branched pre-mirtron (excised intron)
      - Debarking
      - Trimming
    - or
      - pre-miRNA
MECHANISMS OF miRNA-MEDIATED TRANSLATIONAL DOWN-REGULATION

1. INTERFERENCE WITH THE TRANSLATIONAL MACHINERY

Inhibition of translational initiation by interfering with eIF4E binding

Post-initiation blockage of (polysome associated)

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Post-initiation blockage of (polysome associated)
MECHANISMS OF miRNA-MEDIATED TRANSLATIONAL DOWN-REGULATION

2. microRNA SEQUESTRATION OR DEGRADATION INTO P-BODIES

From yeast to mammals a major route for mRNA degradation proceeds in cytoplasmic P-bodies:

- Main Components of P-bodies
- Decapping and desadenylating enzymes and cofactors
- Exonucleases
- Ago proteins
- Untranslated mRNA

GENE-EXPRESSION PROFILES (cDNA microarrays)

can only be used to interpret cellular changes that affect mRNA synthesis but not real protein levels

MicroRNA PROFILING

microRNA profiling expression provides information about translation efficiency of transcripts detected by cDNA microarrays

PROTEOMICS

It should identify the real profile of proteins including their post-translational modifications
Complementarity of seven or more bases to the 5’ end miRNA is sufficient to confer regulation.

Sites with weaker 5’ complementarity require compensatory pairing to the 3’ end to be functional.

Extensive pairing to the 3’ end of without 5’ pairing is not functional.

GENOME–WIDE ANALYSIS OF miRNA CONTROL

- miRNAs are estimated to comprise ~ 1-2% of animal genes (most abundant classes of regulators of gene expression)

- ~ 20 to 30% of all human genes are targets of miRNA regulation (100-200 mRNA per miRNA)

Selective pressure to avoid miRNA regulation segregates mRNAs into

"ANTITARGETS"
Involved in basic processes common to all cells

"TARGETS"
Involved in developmental timing, cell proliferation, apoptosis, metabolism, cell differentiation and morphogenesis
miRNAs HAVE HAD A PROFOUND IMPACT IN 3’ UTR EVOLUTION BY AVOIDANCE AND ENRICHMENT OF TARGET SITES

Table 1. miRNA Target and Antitarget Categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th># Genes</th>
<th>p(over) in Targets</th>
<th>p(under) in Antitargets</th>
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<tr>
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<td>Organogenesis</td>
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<td>p(under) in Targets</td>
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</table>

miRNA-1
EXCEPTIONAL MUSCULARITY OF THE BELGIAN TEXEL SHEEP

- A point mutation that creates an illegitimate microRNA target site in the 3' UTR of *myostatin*

- Inhibits its expression and contributes to the muscular hypertrophy of Texel sheep.
MicroARNs Y CÁNCER
The microRNA-10b is highly expressed in metastatic breast cancer cells and positively regulates cell migration and invasion.

Over-expression of miR-10b initiates robust invasion and metastasis.

Inhibition of miR-10b prevents metastasis.
Para desarrollar metástasis óseas o pulmonares las células del tumor primario deben perder la expresión de ciertos microRNA (miR-126 y miR-335)

Niveles de expresión de miR-335 y miR-126 en pacientes con tumores mamarios y su relación con tiempo libre de metástasis.
microRNA THERAPEUTICS
Antagomirs: a new niche for antisense nucleic acids

Delivery of sRNA for therapy
Silencing of microRNAs in vivo with ‘antagomirs’

Jan Krützfeldt, Nikolaus Rajewsky, Ravi Braich, Kallanthottathil G. Rajeev, Thomas Tuschi, Muthiah Manoharan & Markus Stoffel

- Reduced Plasma cholesterol >40%
- Lasting for 20 days
- Cholesterol biosynthesis
- Useful in Hepatic carcinoma and Hepatitis C

Figure 1 | Specific targeting of miR-122 in mouse liver by tail-vein injection of chemically modified single-stranded RNAs. a, Northern blots of total RNA

Antisense 2’-O-methyl (20-OMe) oligoribonucleotides cholesterol conjugation for delivery in vivo