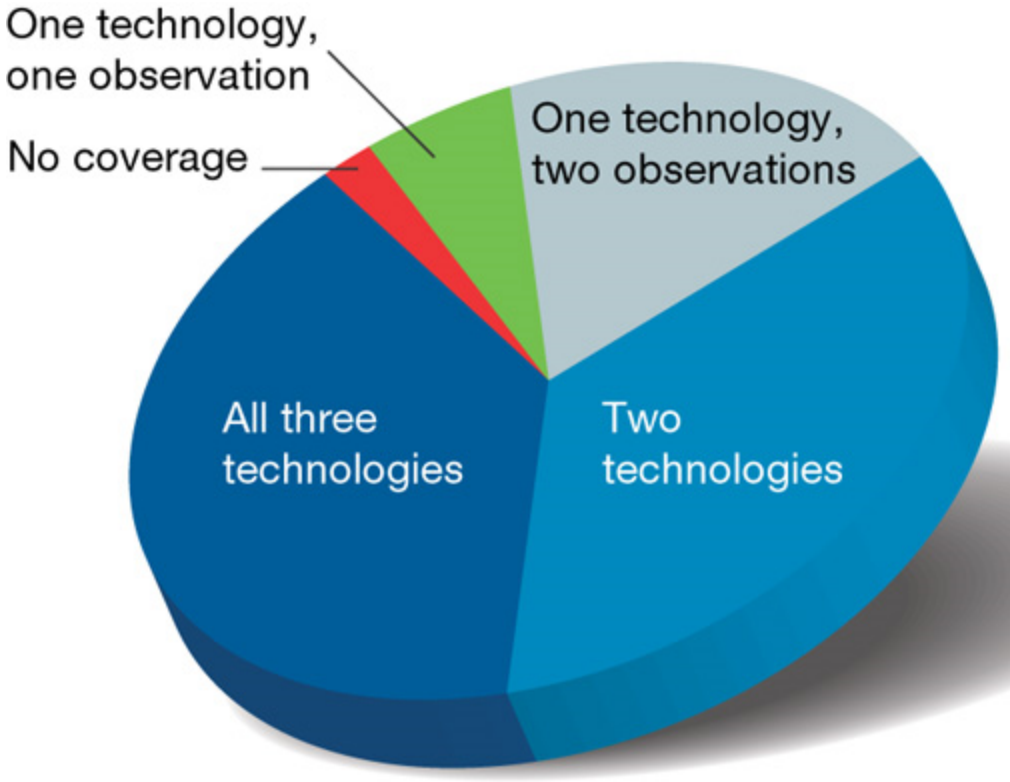
Introduction to miRNA

RNA world hypothesis

The **RNA world hypothesis** proposes that a world filled with life based on ribonucleic acid (RNA) predates the current world of life based on deoxyribonucleic acid (DNA) and protein. The RNA world is proposed to have evolved into the DNA and protein world of today. DNA, through its greater chemical stability, took over the role of data storage while protein, which is more flexible in catalysis through the great variety of amino acids, became the specialized catalytic molecules.

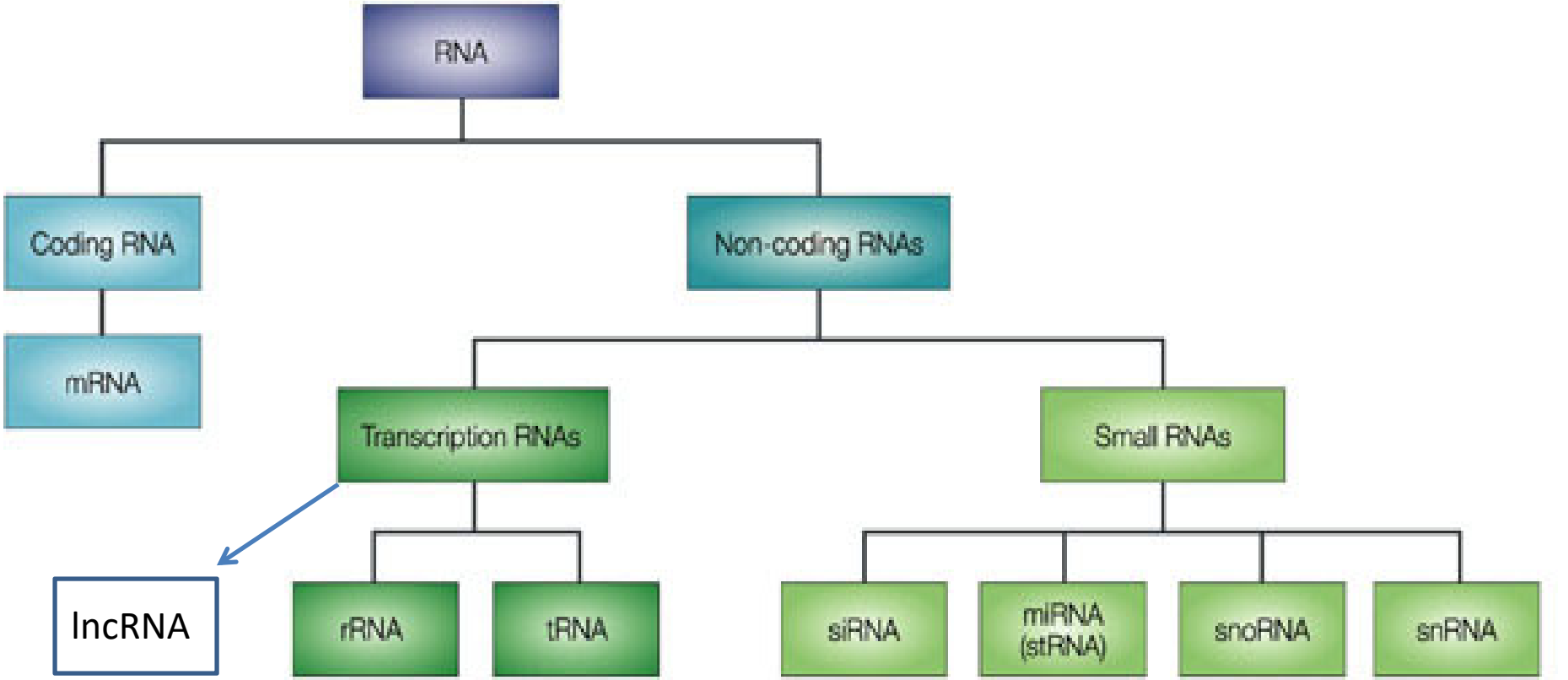
The majority of the human genome is transcribed



93% of bases are represented in a primary transcript identified by at least two independent observations

Nature **447**, 799-816

Tree of RNA Types



Long non-coding RNAs

various transport & structural functions

Protein synthesis

various regulatory roles at the transcriptional level and post-transcriptional level

Process rRNAs

spliceosome

What is miRNA?

MicroRNAs (miRNAs) are a family of ~22 (21 to 25) nucleotide long small RNAs that bind to complementary sequences in the 3’ UTR of multiple target mRNAs, usually resulting in their silencing.

MicroRNAs target ~60% of all genes, are abundantly present in all human cells and are able to repress hundreds of targets each.

miRNAs are conserved in organisms ranging from viruses to the unicellular algae, even to mitochondria, suggest their functions are a vital part of genetic regulation stemming from an ancient origin.

The discovery of miRNAs

- The founding member of the miRNA family, *lin-4*, was identified in *C. elegans*
- In *C. elegans*, cell lineages have distinct characteristics during 4 different larval stages (L1–L4). Mutations in *lin-4* disrupt the temporal regulation of larval development, causing L1 (the first larval stage)- specific cell-division patterns to reiterate at later developmental stages. Opposite developmental phenotypes — omission of the L1 cell fates and premature development into the L2 stage — are observed in worms that are deficient for *lin-14*

lin-4 vs. *lin-14*

- Most genes identified from mutagenesis screens are protein-coding, but *lin-4* encodes a 22-nucleotide non-coding RNA that is partially complementary to 7 conserved sites located in the 3'-untranslated region (UTR) of the *lin-14* gene

lin-4 vs. *lin-14*

- *lin-14* encodes a nuclear protein, downregulation of which at the end of the first larval stage initiates the developmental progression into the second larval stage. The negative regulation of lin-14 protein expression requires an intact 3'UTR of its mRNA, as well as a functional *lin-4* gene
- The direct, but imprecise, base pairing between *lin-4* and the *lin-14* 3' UTR was essential for the ability of *lin-4* to control lin-14 expression through the regulation of protein synthesis

let-7 vs *lin-41&lin-57*

- In 2000, almost 7 years after the initial identification of *lin-4*, the second miRNA, *let-7*, was discovered in worms.
- *let-7* encodes a temporally regulated 21-nucleotide small RNA that controls the developmental transition from the L4 stage into the adult stage. Similar to *lin-4*, *let-7* performs its function by binding to the 3' UTR of *lin-41* and *hbl-1* (*lin-57*), and inhibiting their translation

let-7 and *lin-41*

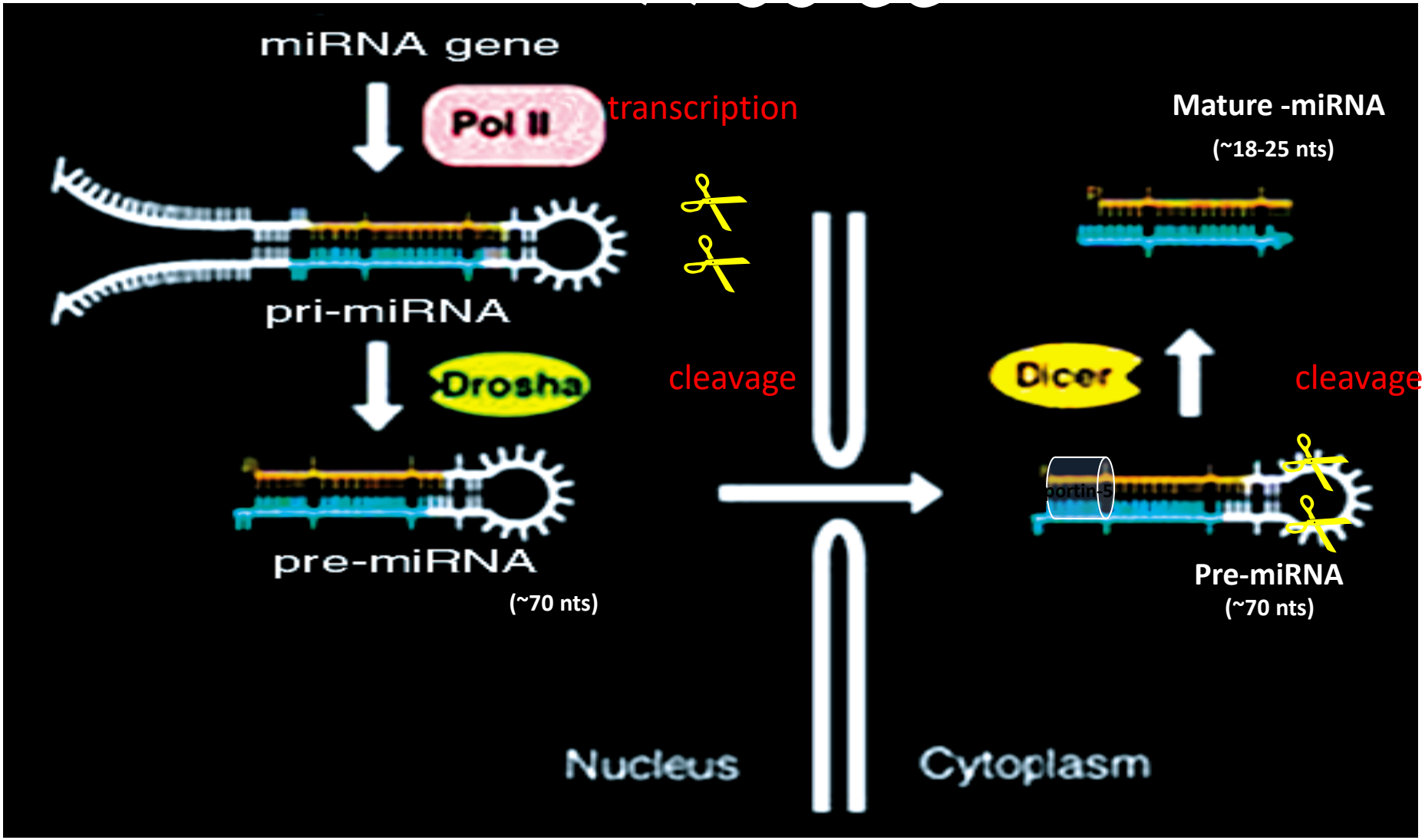
- *let-7* and *lin-41* are evolutionarily conserved throughout metazoans,with homologues that were readily detected in sea urchins, flies, mice and humans, this conservation strongly indicated a more general role of small RNAs in developmental regulation in many metazoan organisms.

Current status

- Since 2000, over 28645 miRNAs have been discovered in all studied eukaryotes including mammals, viruses, fungi and plants, with ~1800 in humans (http://www.mirbase.org/) already identified.

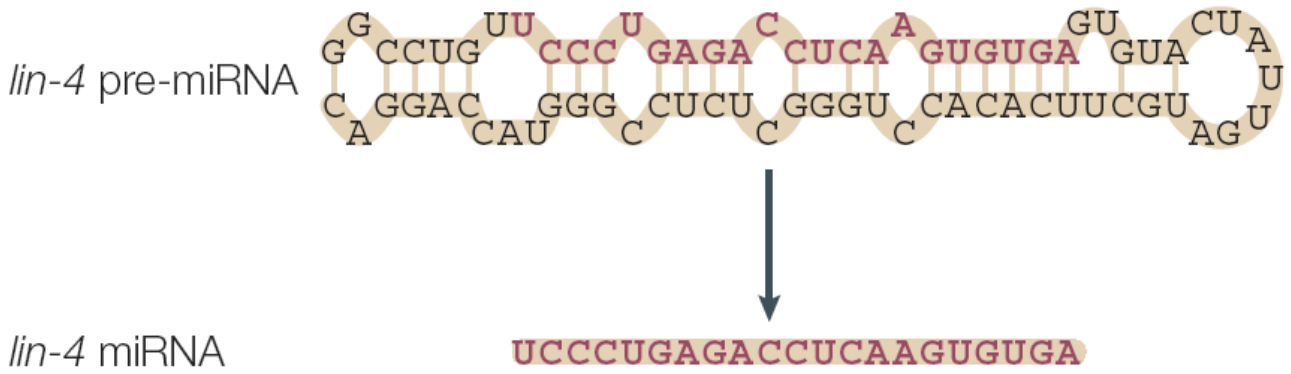
- Comparing miRNAs between species can even be used to delineate molecular evolutionary history on the basis that the complexity of an organisms phenotype may reflect that of the microRNA found in the genotype.

Biogenesis of miRNAs (miR)



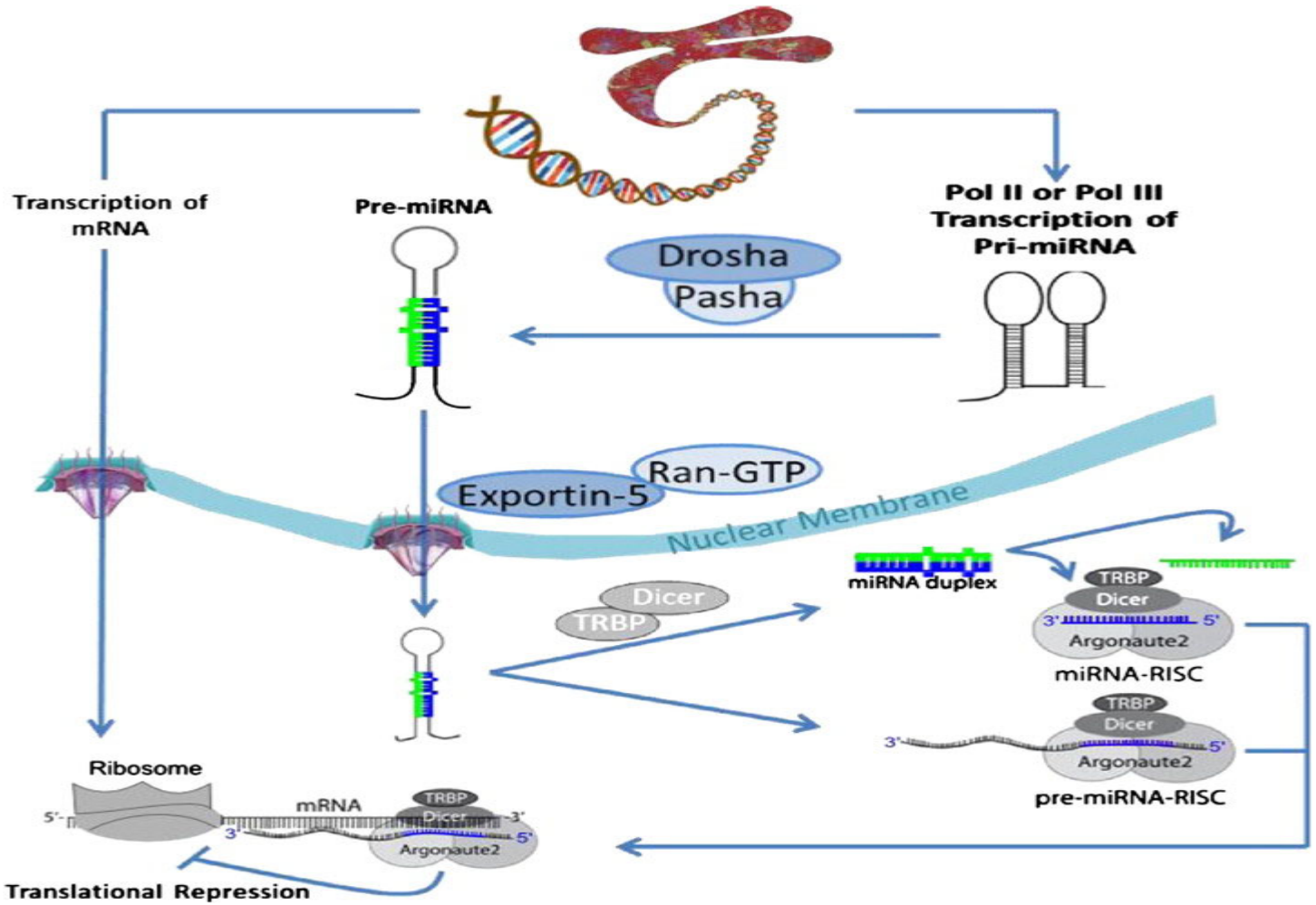
Cell 116:281-297, 2004.

Biogenesis of miRNA: an example



- miRNAs are generally 21–25nucleotide, non-coding RNAs that are derived from larger precursors that form imperfect stem-loop structures, the mature miRNA is most often derived from one arm of the precursor hairpin, and is released from the primary transcript through stepwise processing by two ribonuclease-III (RNase III) enzymes

Biogenesis of miRNAs (miR) (cont')



Genomics. 2011 Jan;97(1):1-6

miRNA Function

- Following the identification of hundreds of miRNAs in various organisms, large-scale studies on miRNA expression profiles were carried out in many model organisms using northern-blot analysis, microarrays and miRNA cloning.

- miRNAs show dynamic temporal and spatial expression patterns, disruption of which is associated with developmental and/or physiological abnormalities.

- These findings indicate that miRNAs might have a general role in regulating gene expression in diverse developmental and physiological processes, and provide substantial hints that misregulation of miRNA function might contribute to human disease

miRNA Function

- Most of the miRNAs that have been characterized so far seem to regulate aspects of development, including larval developmental transitions and neuronal development in *C. elegans*, growth control and apoptosis in *D. melanogaster*, haematopoietic differentiation in mammals and leaf development in *A. thaliana.*

miRNA Function

- But, only a handful of miRNAs have been carefully studied so far, and the diverse expression patterns of miRNAs and altered miRNA expression under certain physiological conditions, such as tumorigenesis, indicate a range of unknown functions that might extend beyond developmental regulation.

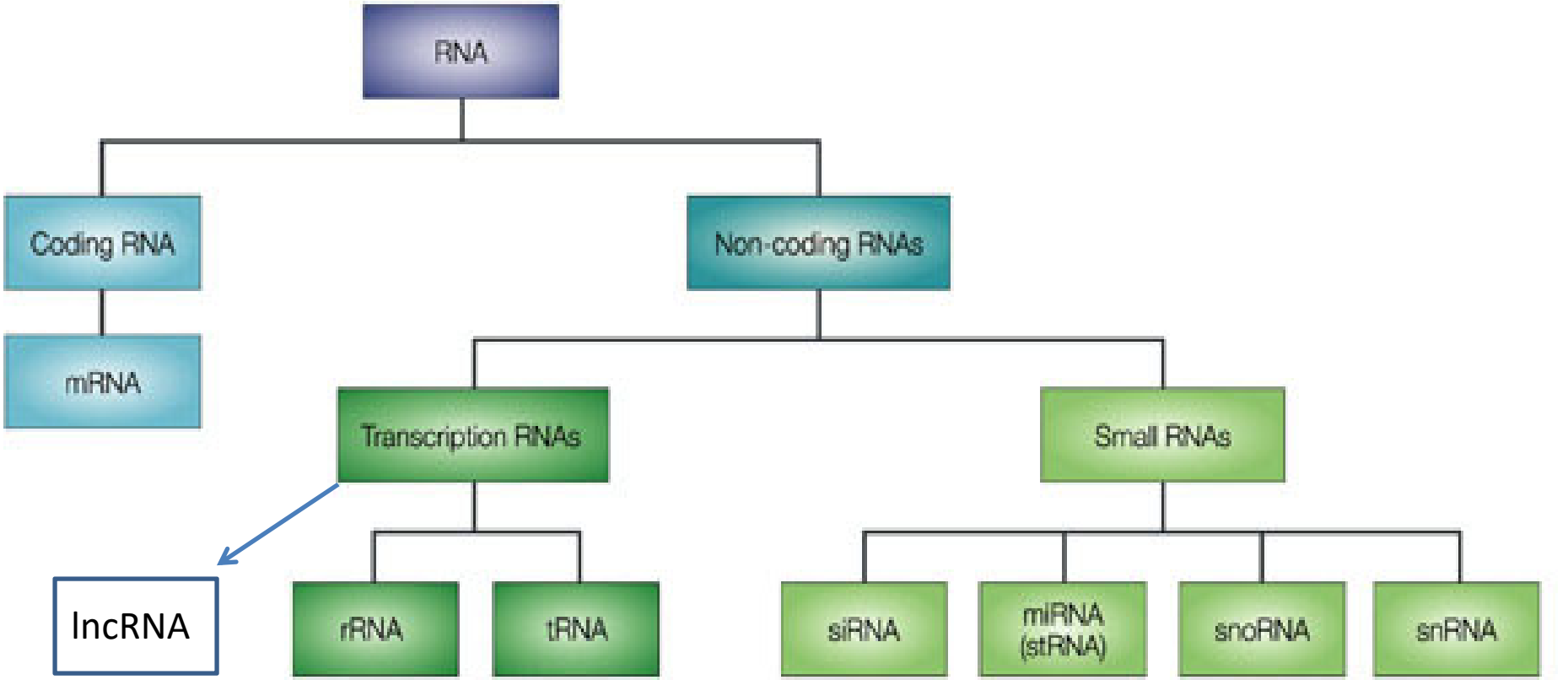
- In addition, bioinformatic predictions of miRNA targets have revealed a diversity of regulatory pathways that might be subject to miRNA-mediated regulation.

- Overall, no specific indication has emerged that miRNA-mediated regulation is restricted to one biological process. Instead, the emerging picture is that miRNAs have the potential to regulate almost all aspects of cellular physiology.

Function: conclusion

Due to their abundant presence and far-reaching potential, miRNAs have all sorts of functions in physiology, from cell differentiation, proliferation, apoptosis to the endocrine system, haematopoiesis, fat metabolism, limb morphogenesis. They display different expression profiles from tissue to tissue, reflecting the diversity in cellular phenotypes and as such suggest a role in tissue differentiation and maintenance.

Tree of RNA Types



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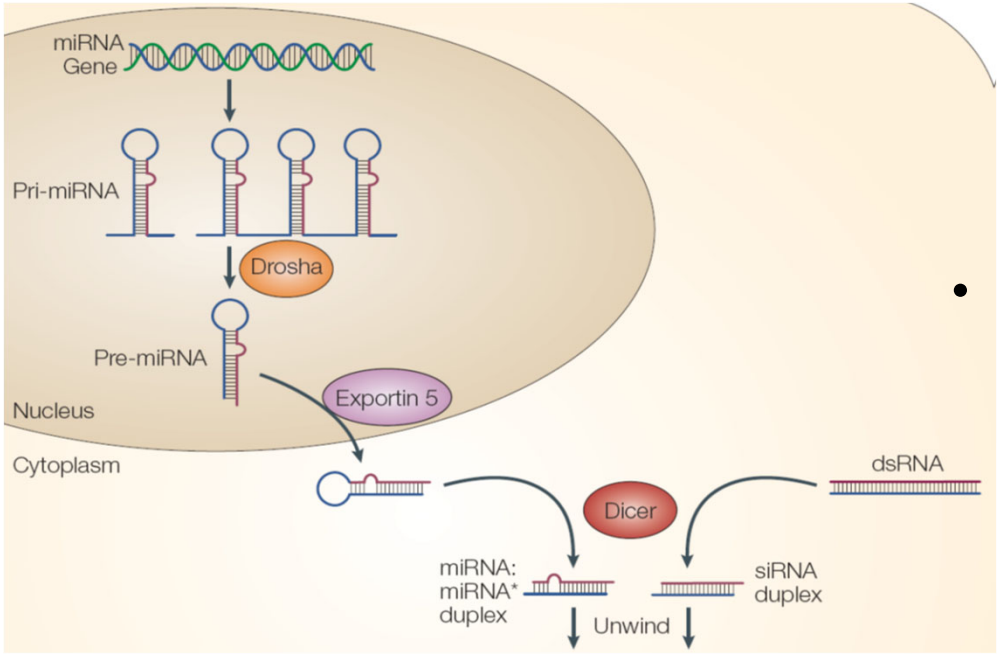
miRNA vs siRNA-Similarities

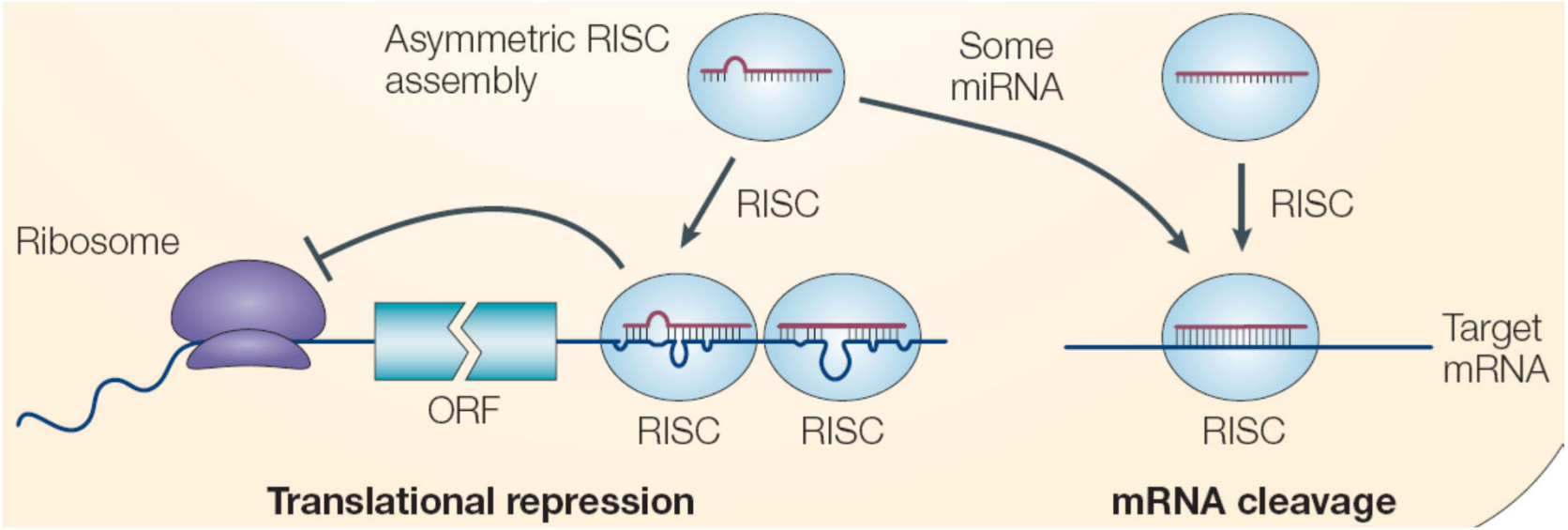
- miRNAs and siRNAs share a common RNase-III processing enzyme, Dicer, and closely related effector complexes, RISCs, for post-transcriptional repression.

- siRNAs and miRNAs are similar in terms of their molecular characteristics, biogenesis and effector functions. So, the current distinctions between these two species might be arbitrary, and might simply reflect the different paths through which they were originally discovered.

miRNA vs siRNA-Differences



- miRNAs differ from siRNAs in their molecular origins and, in many of the cases that have been characterized so far, in their mode of target recognition.
- miRNAs are produced as a distinct species from a specific precursor that is encoded in the genome. By contrast, siRNAs are sampled more randomly from long dsRNAs that can be introduced exogenously or produced from bi-directionally transcribed endogenous RNAs that anneal to form dsRNA.



- In many cases, miRNAs bind to the target 3' UTRs through imperfect complementarity at multiple sites, and therefore negatively regulate target expression at the translational level. By contrast, siRNAs often form a perfect duplex with their targets at only one site, and therefore direct the cleavage of the target mRNAs at the site of complementarity.

More…

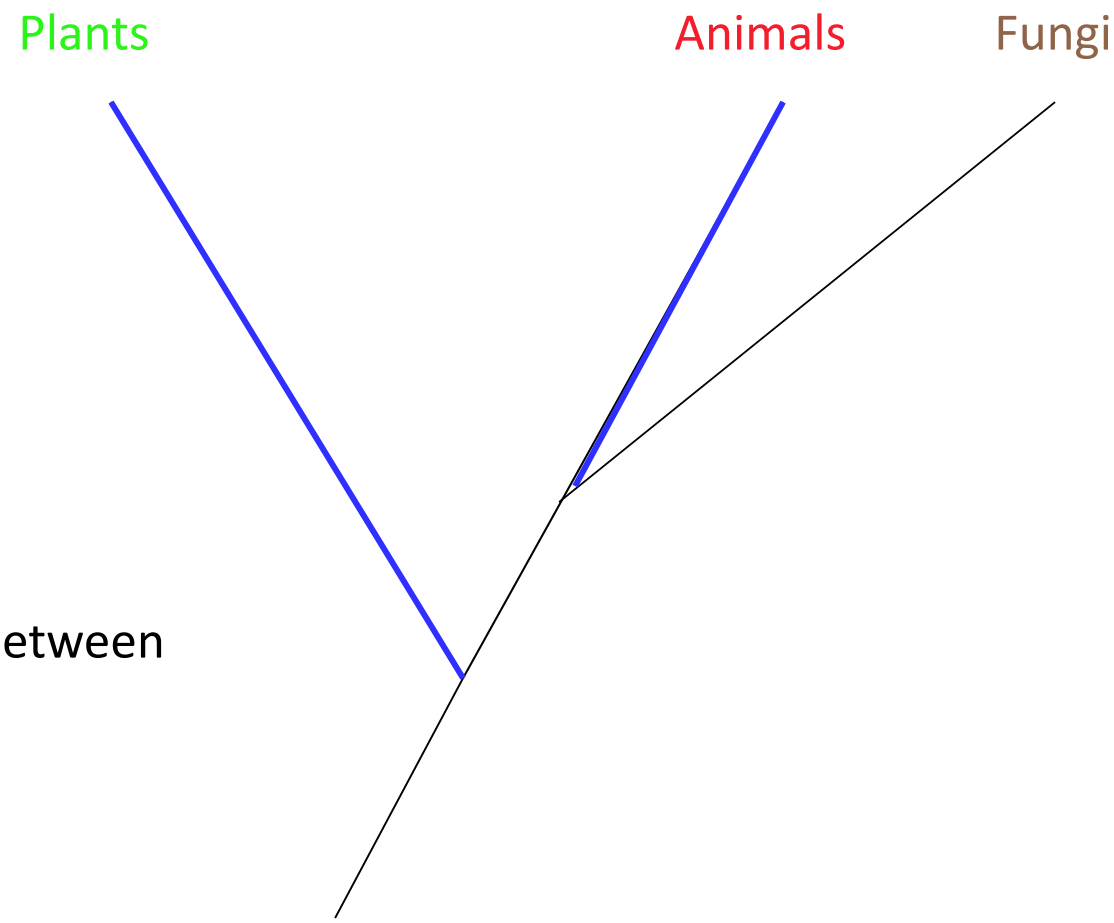
The extent of complementarity between the siRNA/miRNA and its target can determine the mechanism of silencing.

- With the exception of *miR-172*,which acts as a translational repressor, all characterized plant miRNAs anneal to their targets with nearly complete complementarity at a single site, either in the coding region or in the UTRs, therefore condemning their target mRNAs to destruction by cleavage and degradation.
- A similar situation has also been described for one mammalian miRNA, *miR-196*: the nearly perfect base pairing between *miR-196* and *Hoxb8* directs cleavage of *Hoxb8* mRNA both in mouse embryos and in cell culture.
- Conversely, when siRNAs pair with their targets imperfectly, siRNAs can trigger translational repression rather than mRNA cleavage in mammalian tissue culture.
- So, we are left with the question of whether miRNAs are truly different from siRNAs or whether our current understanding fails to functionally distinguish these two species under physiological conditions

miRNA in animal and plant

- Plant miRNAs differ from animal miRNAs in that their base pairing with the corresponding targets is nearly perfect, and that their complementary sites are located throughout the transcribed regions of the target gene, instead of being limited to the 3’UTRs

Evidence suggests miRNAs evolved independently in plants and animals



miRNAs absent from fungi

General lack of homology between plant and animal miRNAs

miRNA clusters arose from gene duplication events

In plants, duplication event(s) happened more recently:

Clustered miRNAs have more sequence homology

Gene dosage effect

In animals, duplication event(s) happened earlier:

Clustered miRNAs have more diverged sequence

Clustered miRNAs have partially redundant target specificity

Methods for miRNA discovery

1. Generation of small RNA libraries

    Isolate small RNAs on polyacrylamide gel

    Make cDNA library (random primers)

    High throughput sequencing

Advantages/Limitations

    +Potential to find new miRNAs

    -Technically challenging and expensive procedure

    -A portion of small RNAs are degredation products

Methods for miRNA discovery

2. Hybridization based approaches

MicroRNA microarrays are commercially available

Advantages/Limitations

+Current technologies can decrease variability in Tm and increase stringency of probes

+Don’t need small RNA enrichment

-Limited to known miRNAs

Alternative methods for miRNA discovery

3. Computational identification of miRNA genes

Use homology searches to ID miRNAs in new species

Search for stem loops in vicinity of known miRNAs

ID conserved motifs in 3'UTRs

Distinctive conservation pattern

Advantages/Limitations

+Quick and dirty search good for preliminary studies

-Requires extensive experimental validation

-Based on assumptions of clusters and complementarity

A comprehensive miRNA registry

http://www.mirbase.org/

- The miRBase is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase represents a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR). Both hairpin and mature sequences are available for searching and browsing, and entries can also be retrieved by name, keyword, references and annotation. All sequence and annotation data are also available for download.

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