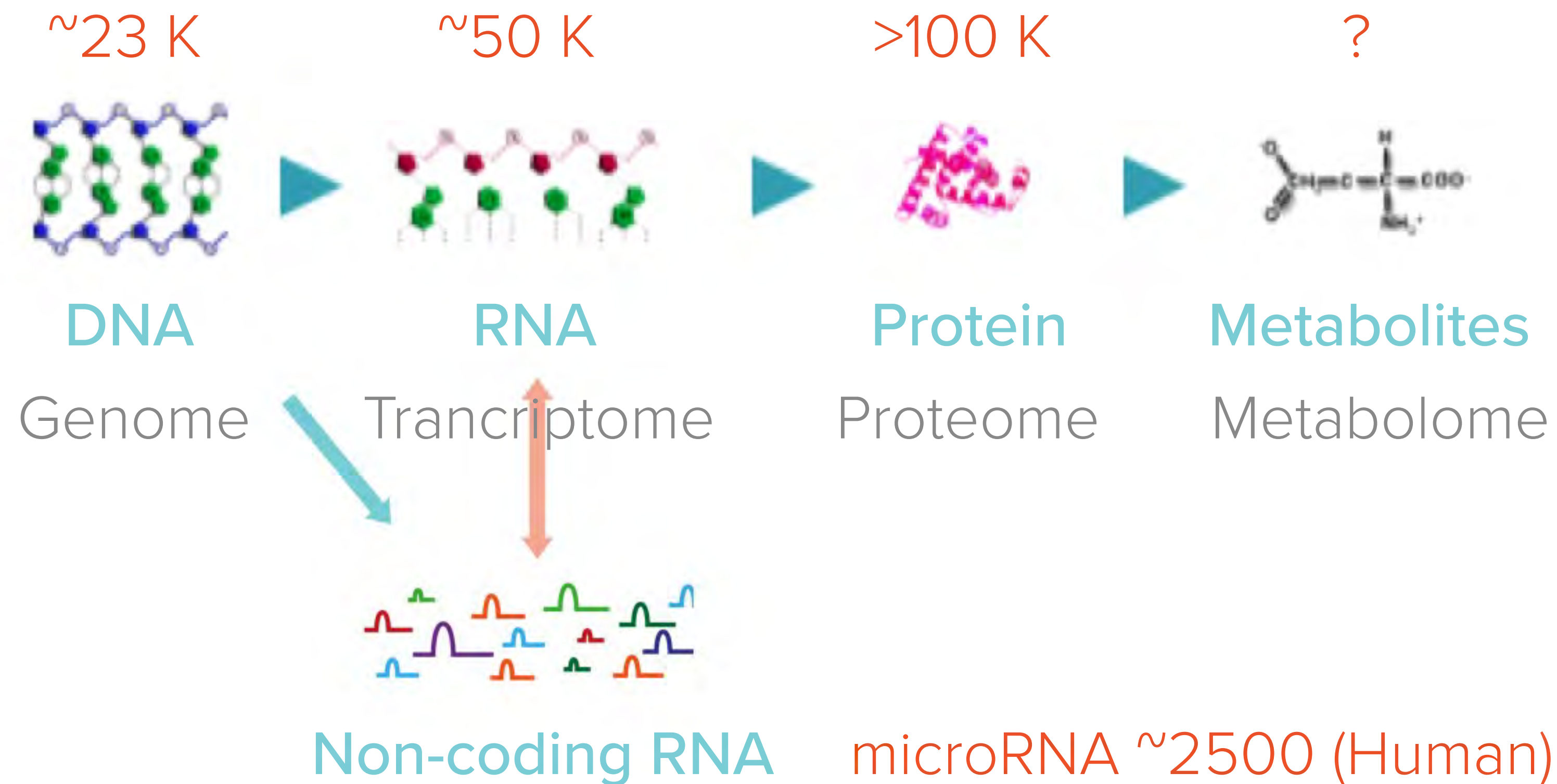


Non-coding RNA – A New Kid of the Block

Central Dogma – revisited



What is microRNA?

- microRNA: short noncoding single stranded RNA
- Mature miRNA is approximately 22 nucleotides in length
- Currently known: 1881 precursors and 2578 mature microRNAs (Human, miRBase 21.0)
- miRBase – Central Depository for microRNA Annotations
<http://www.mirbase.org/>



The screenshot shows the miRBase website interface. At the top, there is a dark blue header with the miRBase logo on the left, the text "miRBase" in the center, and the "MANCHESTER 1824" logo on the right. Below the header is a light blue navigation bar with links: Home, Search, Browse, Help, Download, Blog, and Submit. A search bar is located on the right side of this bar. The main content area is divided into two columns. The left column is titled "Latest miRBase blog posts" and contains two entries. The first entry is titled "High confidence miRNA set available for miRBase 21" and is dated July 3, 2014. The second entry is titled "miRBase 21 finally arrives" and is dated June 26, 2014. The right column contains three sections: "miRNA count: 28645 entries" with a sub-section "Release 21: June 2014", a "Search by miRNA name or keyword" section with a search bar and buttons for "Go" and "Example", and a "Download published miRNA data" section with links for "Download page" and "FTP site".

miRBase

MANCHESTER 1824

Home Search Browse Help Download Blog Submit

Latest miRBase blog posts

High confidence miRNA set available for miRBase 21 By sam (July 3, 2014)
As mentioned previously, we briefly held off from releasing the set of "high confidence" miRNAs for miRBase 21, because of a last-gasp bug. Those data are now available, tagged with the label "high confidence" on the entry pages, and for download on the FTP site. The total number of miRNAs labelled "high confidence" has increased [...]

miRBase 21 finally arrives By sam (June 26, 2014)
Apologies for the longer-than-usual wait. miRBase 21 is now available on the website, and all data available for download on the FTP site. As usual, the release notes describe the major changes. Of particular note this time, the Genome Reference Consortium have released a new human genome assembly, GRCh38. We have therefore remapped the human [...]

miRNA count: 28645 entries
Release 21: June 2014

Search by miRNA name or keyword
 Go Example

Download published miRNA data
[Download page](#) | [FTP site](#)

microRNA biogenesis

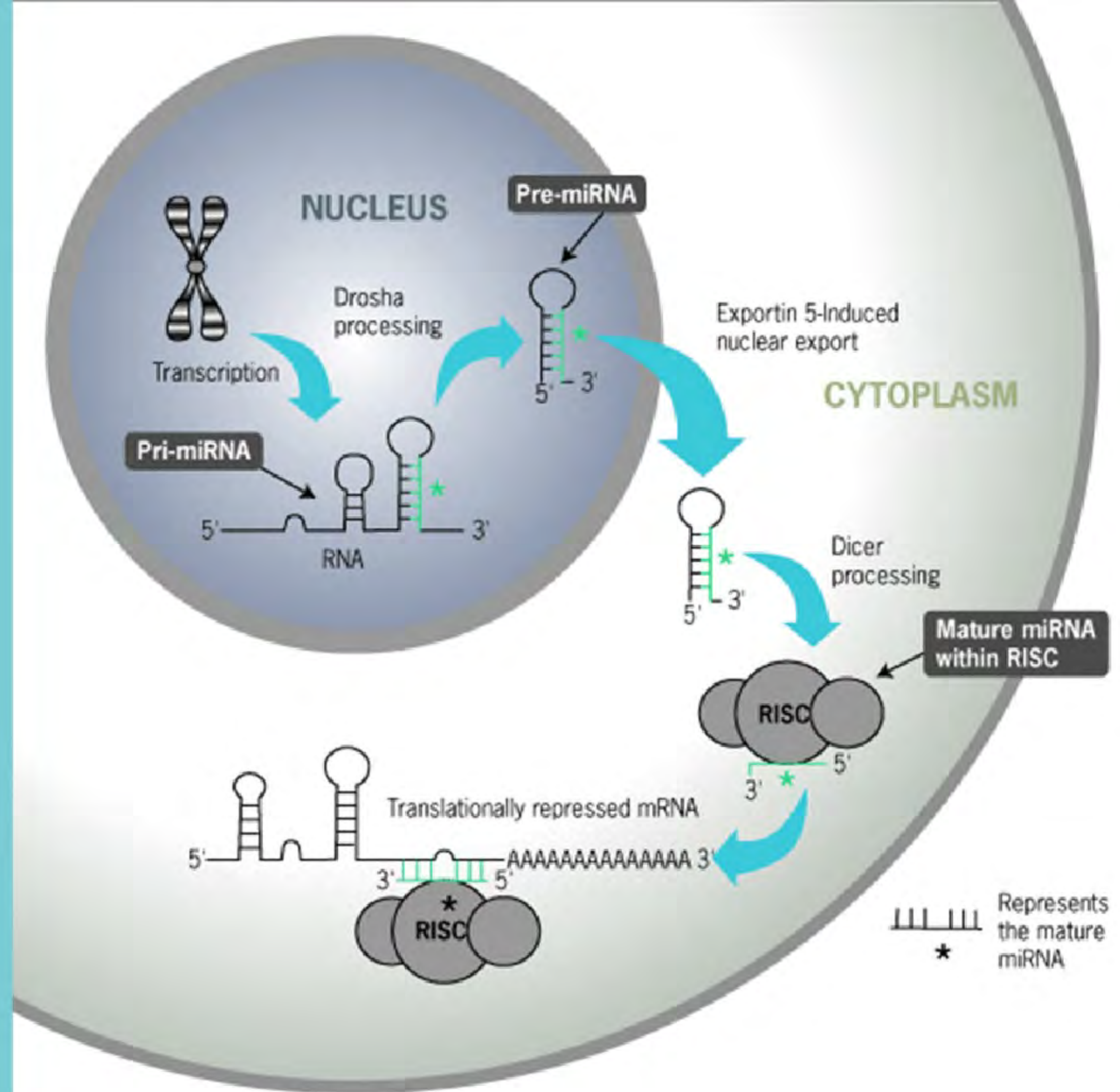
- The genes encoding miRNAs are much longer than the processed mature miRNA molecules.
- Many miRNAs are known to reside in introns of their pre-mRNA host genes and share their regulatory elements, primary transcript, and have a similar expression profile. For the remainder of miRNA genes that are transcribed from their own promoters, few primary transcripts have been fully identified.
- MicroRNAs are transcribed by RNA polymerase II as large RNA precursors called pri-miRNAs and comprise of a 5' cap and poly-A tail.

microRNA biogenesis

- The pri-miRNAs are processed in the nucleus by the microprocessor complex, consisting of the RNase III enzyme Drosha, and the double-stranded-RNA-binding protein, Pasha/DGCR8. The resulting pre-miRNAs are approximately 70-nucleotides in length and are folded into imperfect stem-loop structures.
- The pre-miRNAs are then exported into the cytoplasm by the karyopherin exportin 5 (Exp5) and Ran-GTP complex.
- Once in the cytoplasm, the pre-miRNAs undergo an additional processing step by the RNase III enzyme Dicer generating a mature miRNA, a single-stranded RNA approximately 22 nucleotides in length.
- Dicer also initiates the formation of the RNA-induced silencing complex (RISC).

microRNA

Biogenesis and Function



microRNA Function

Major function of microRNA

Post-transcriptionally regulate gene expression in plants and animals

2 major mechanisms of action of miRNAs in post-transcriptional gene regulation

- 1 • Inhibition of translation initiation via complementary elements in the 3 UTRs of their target mRNAs
- 2 • mRNA degradation

microRNA Function

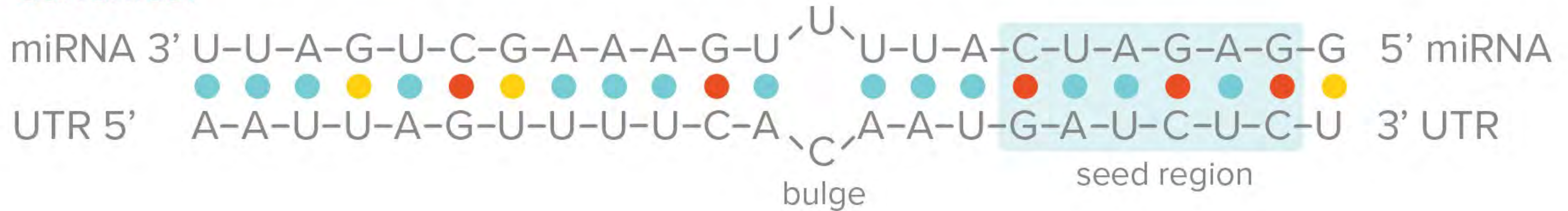
Important regulatory role: “Roomkeeping genes”

- developmental timing
- cellular differentiation, proliferation, apoptosis
- insulin secretion, and cholesterol biosynthesis
- moderating immune response
- oncogenesis

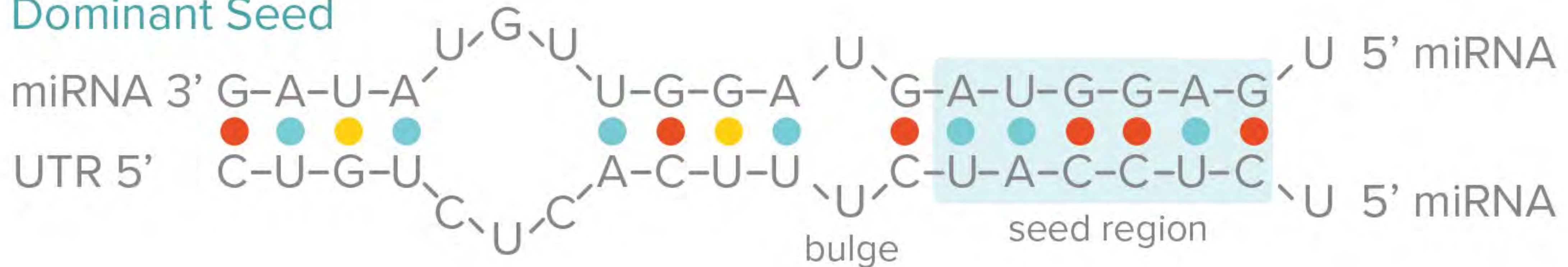
microRNA

Target Interactions

Canonical



Dominant Seed

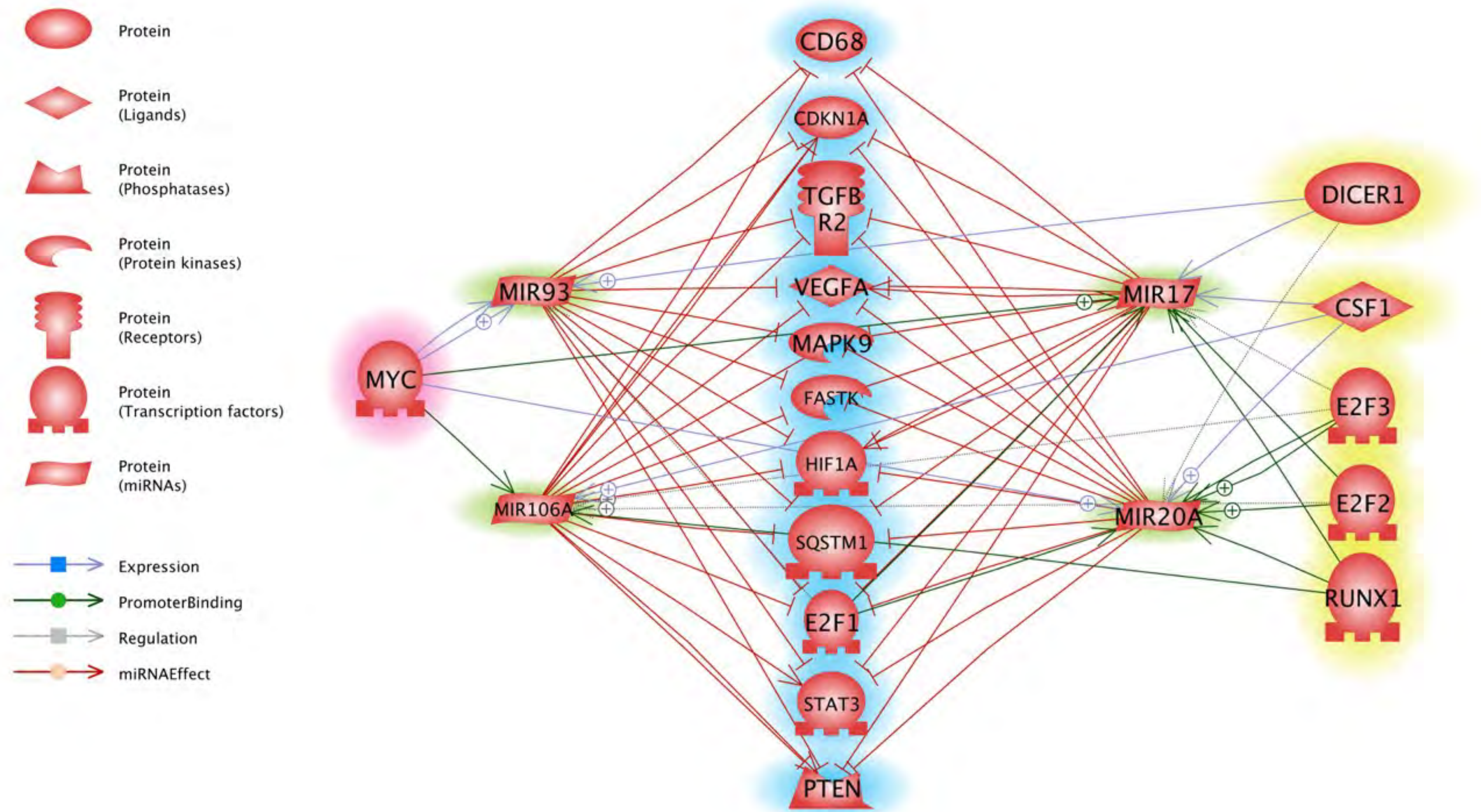


Tools for Target Prediction / Identification

Name	Type	URL	Classification
miRanda	L	http://www.microrna.org/microrna/getMirnaForm	Seed-based
TargetScan	W	http://www.targetscan.org/	
Diana-MicroT	W	http://diana.cslab.ece.ntua.gr/microT/	
RNAHybrid	W/D	http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/	
PicTar	L	http://pictar.mdc-berlin.de/	
TargetBoost	W	https://demo1.interagon.com/targetboost/	Machine learning
miTarget	W	http://cbit.snu.ac.kr/&#24;miTarget/	
mirWip	L	http://mirtargets.org	Target structure integration
MicroTar	D	http://tiger.dbs.nus.edu.sg/microtar/	

W: Webserver D: Downloadable program L: List of predictions

Example: The miR-17/92 cluster



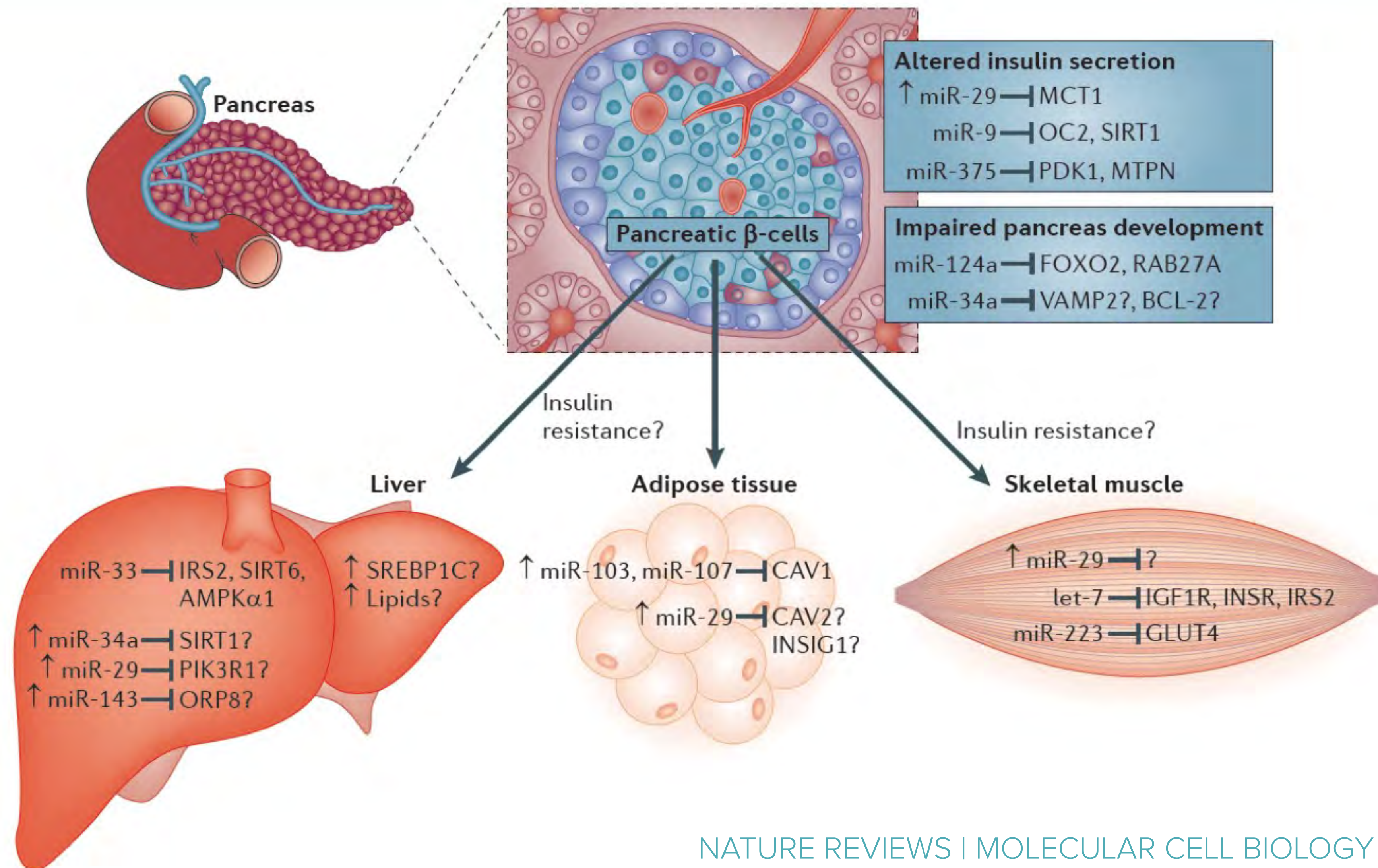
- Experimentally validated targets (blue highlights) and regulators (yellow and red highlights) are shown connected to their corresponding microRNAs (green highlights).
- miR-17/92 cluster is playing increasingly important and numerous roles in health and disease

Role of microRNA in Diseases

microRNA plays important regulatory functions that are organ and tissue specific and **disease specific**

- Metabolism and Metabolic Disorders
- Hematological System and Immune System: development & diseases
- Cardio vascular system: development & diseases
- Central Nervous System & Neurodegenerative Diseases
- Cancer
- And many other organ specific diseases

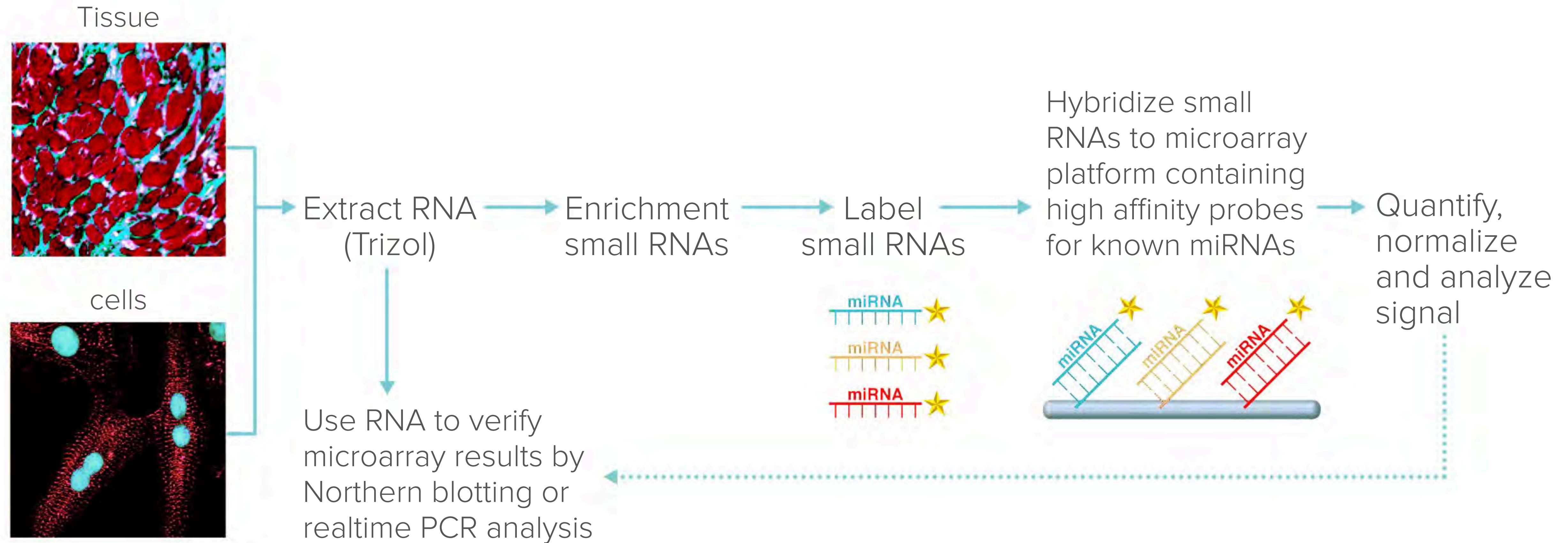
Example: MicroRNA regulation of insulin and glucose homeostasis



Known and predicted targets that lack in vivo evidence are marked with a question mark. In disease conditions, such as impaired insulin secretion or insulin resistance, several miRNAs are upregulated (marked with an arrow).

Microarray analysis of microRNA expression

Eva van Rooij Circ Res. 2011;108:219-234



MiRNA-specific reverse transcription

Eva van Rooij Circ Res. 2011;108:219-234

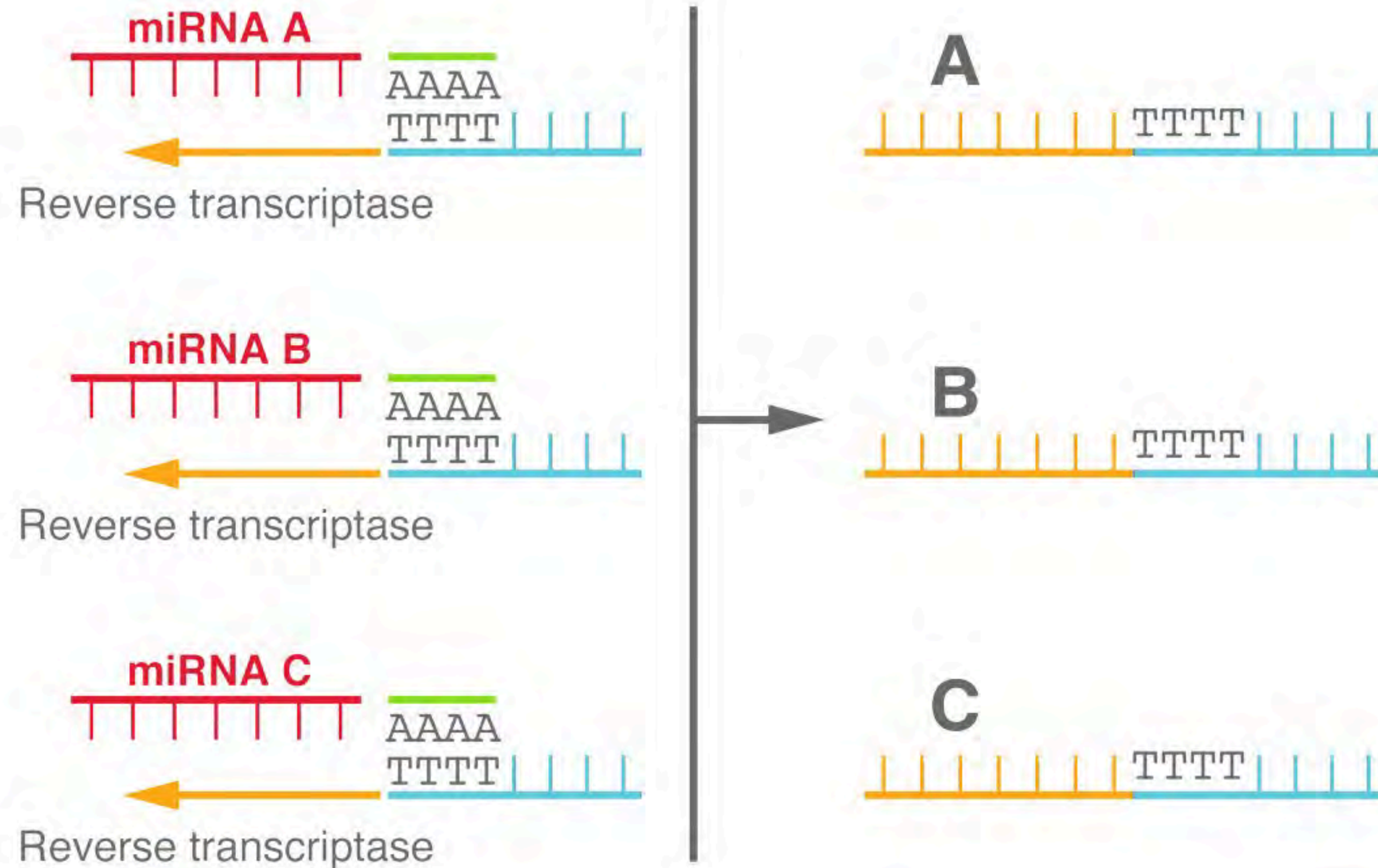
A Stemloop specific primer



MiRNA-specific reverse transcription

Eva van Rooij Circ Res. 2011;108:219-234

B Universal primer



MiRNA biogenesis and research tools

Eva van Rooij Circ Res. 2011;108:219-234

- Microarray analysis for miRNAs
- Deep sequencing
- Realtime PCR
- Northern blotting
- In situ hybridization

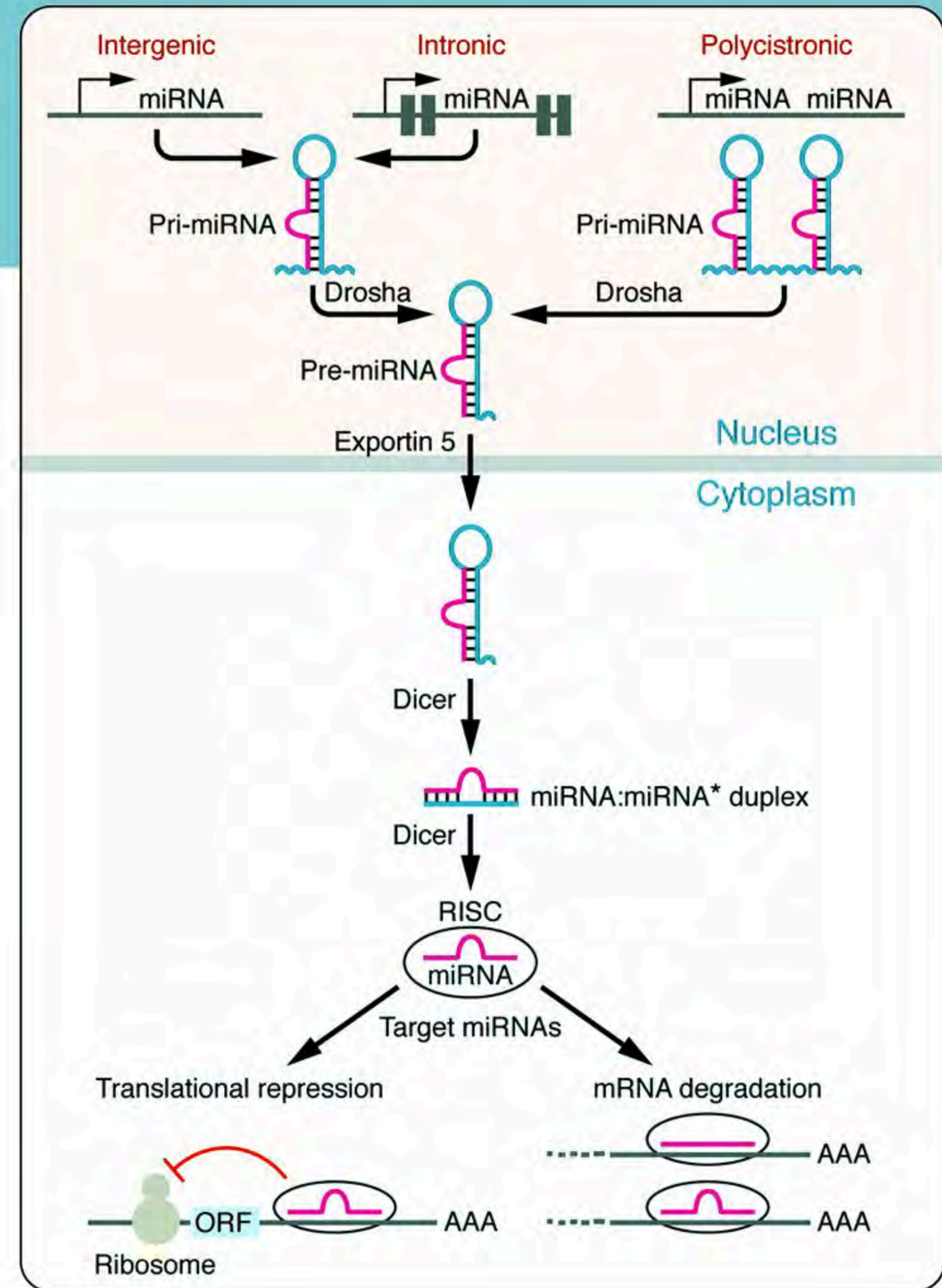
Detection

- Bioinformatics
- UTR analysis
- Transcriptome / proteome analysis
- Pull-down assays

Target determination

- *In vitro* miRNA regulation
- Genetic manipulation of miRNAs
- miRNA inhibition *in vivo*
- miRNA mimicry *in vivo*

Regulation



Opportunities and challenges for microRNA studies

