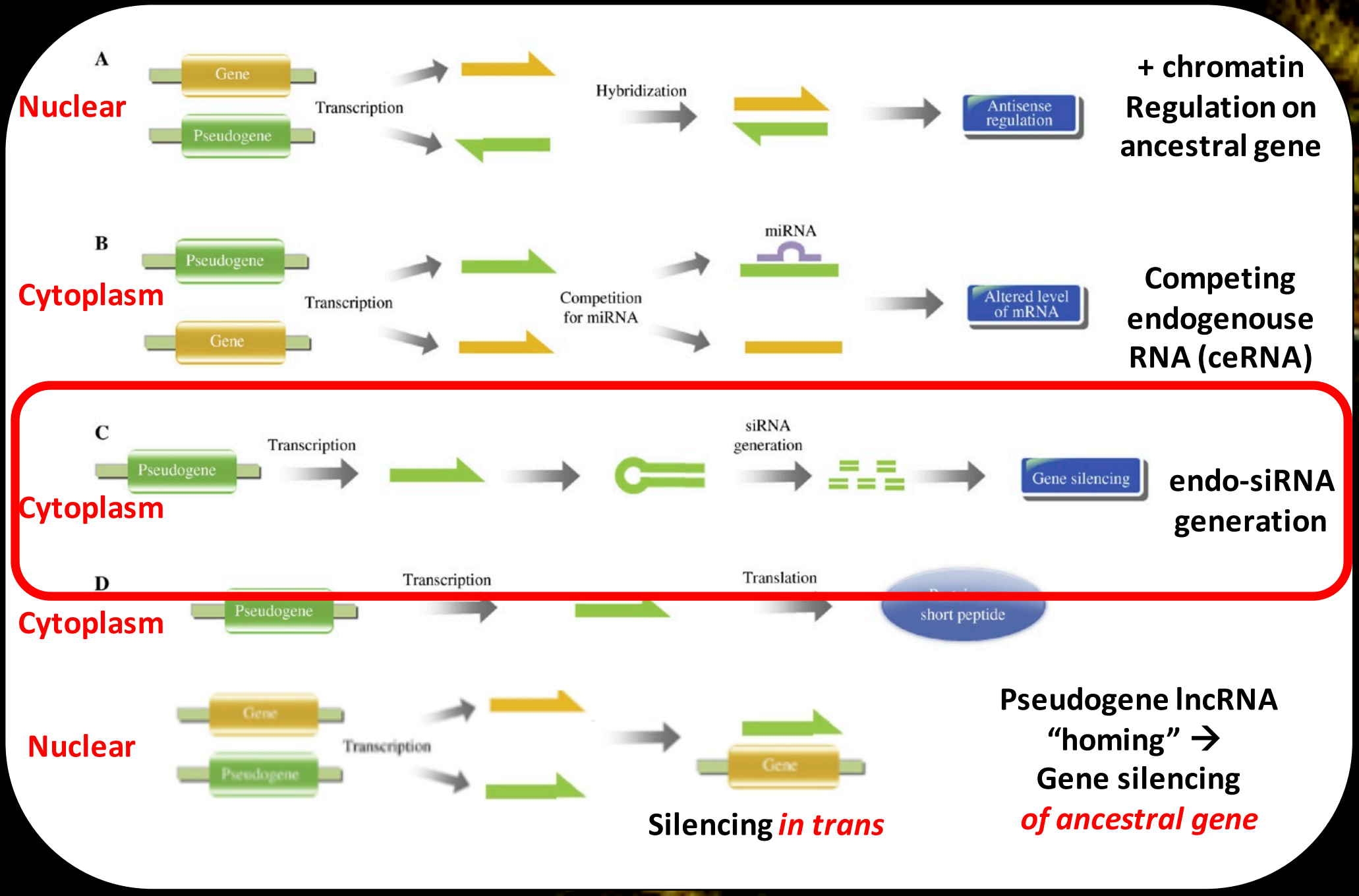
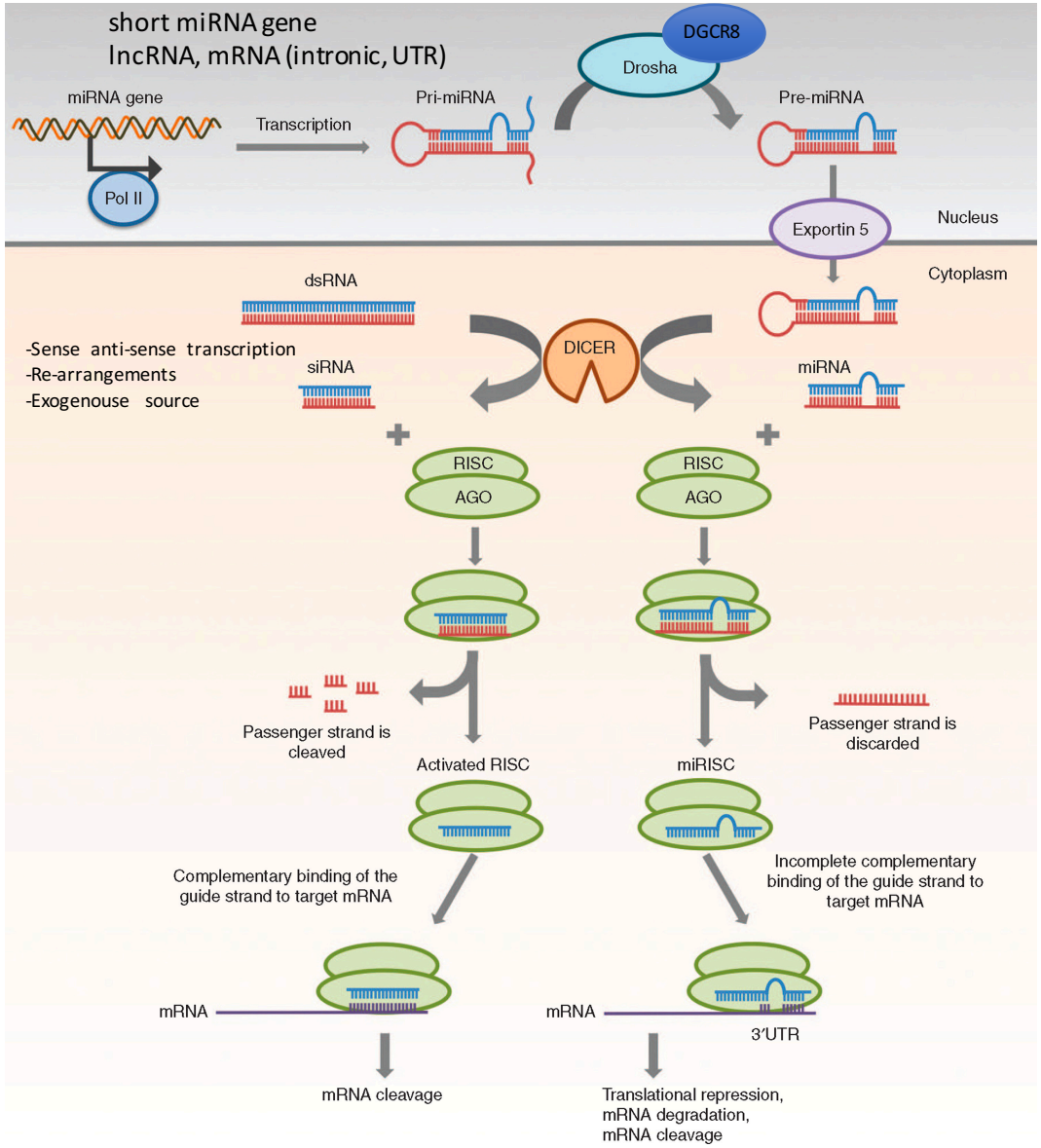
Small RNAs from endogenous loci
endo-siRNAs

Pseudogenes are powerful regulators of gene expression



siRNA and miRNA biogenesis and gene regulation



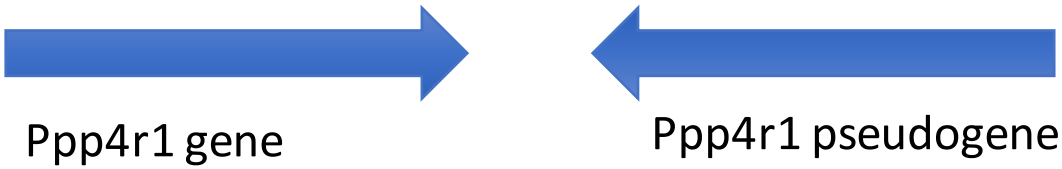
miRNA biogenesis

1. Long, unprocessed precursor dsRNA or stem loop RNA **(pri-miRNA)**
2. Processing in the nucleus by the RNaseIII family protein Drosha generates a stem-loop RNA with characteristic length of 65-70 nucleotides. Drosha is in complex with DGCR8 that is important for Drosha activity
3. Exportin 5-RanGTP transports pre-miRNA in ternary complex thought nuclear pore to cytoplasm. RanGAP stimulates GTP; pre-miRNA released from Exportin.
4. RNaseII family enzyme Dicer processes pre-miRNA generating a 20-25 base dsRNA with overhang at the 3'end (2 bases)
5. Transfer of dsRNA to RISC complex (RNA induced silencing complex)
6. Selection of guide RNA → regulatory RNA passenger RNA → will be eliminated
7. RISC complex+guide RNA → regulatory function

A. RNA degradation = siRNA effect (cutting = "slicing"
B. inhibition of mRNA translation =mRNA effect
C. transfer to nucleus and chromatin regulation = siRNA mediated silencing

Discovery of pseudogene derived endo-siRNAs

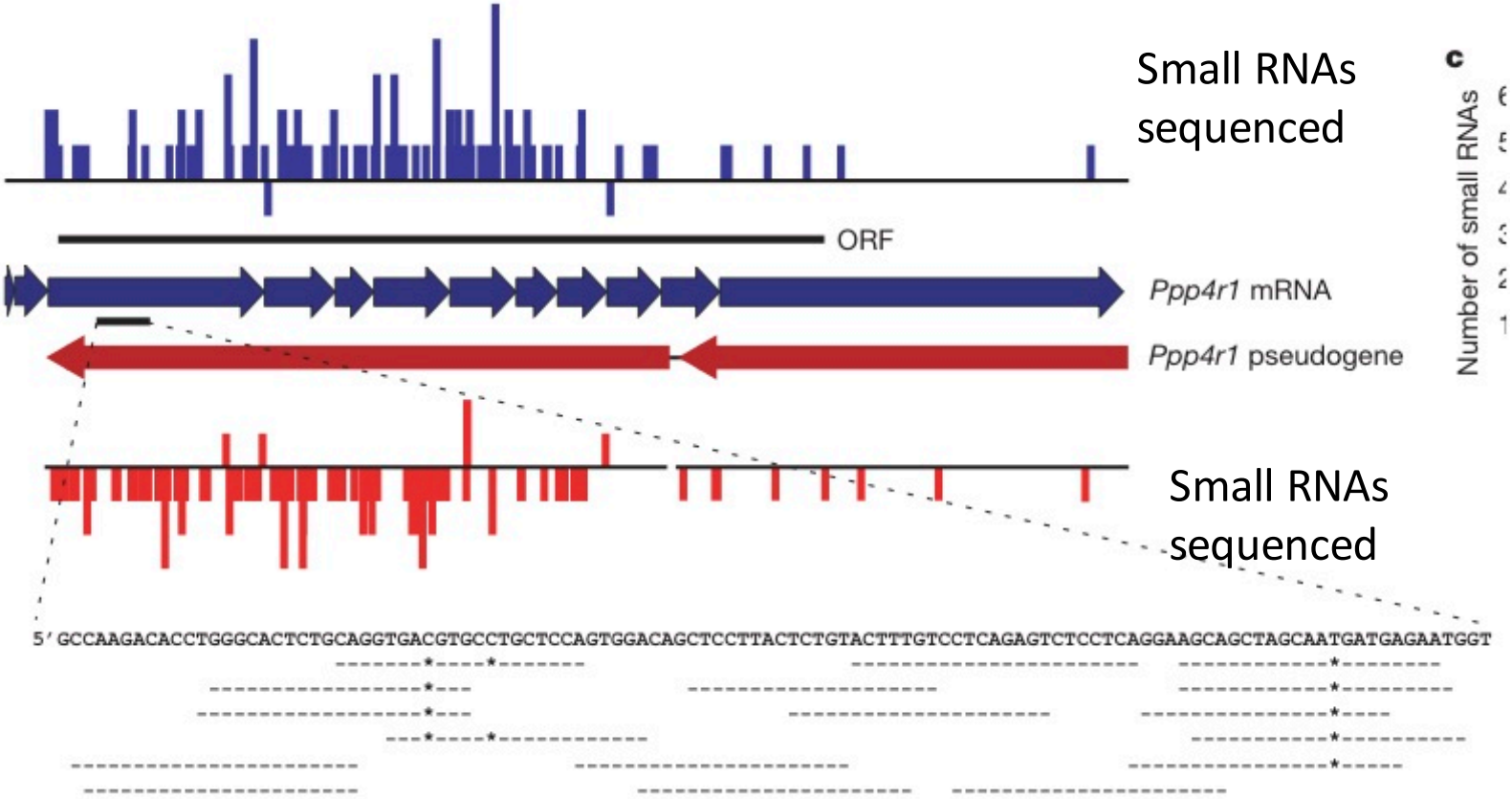
Massive parallel sequencing of small RNAs
A small fraction of RNAs (21-22nt) was found to map to pseudogenes
siRNA map only to regions of complementarity (not to miRNA genes)



Ppp4r1 gene

Ppp4r1 pseudogene

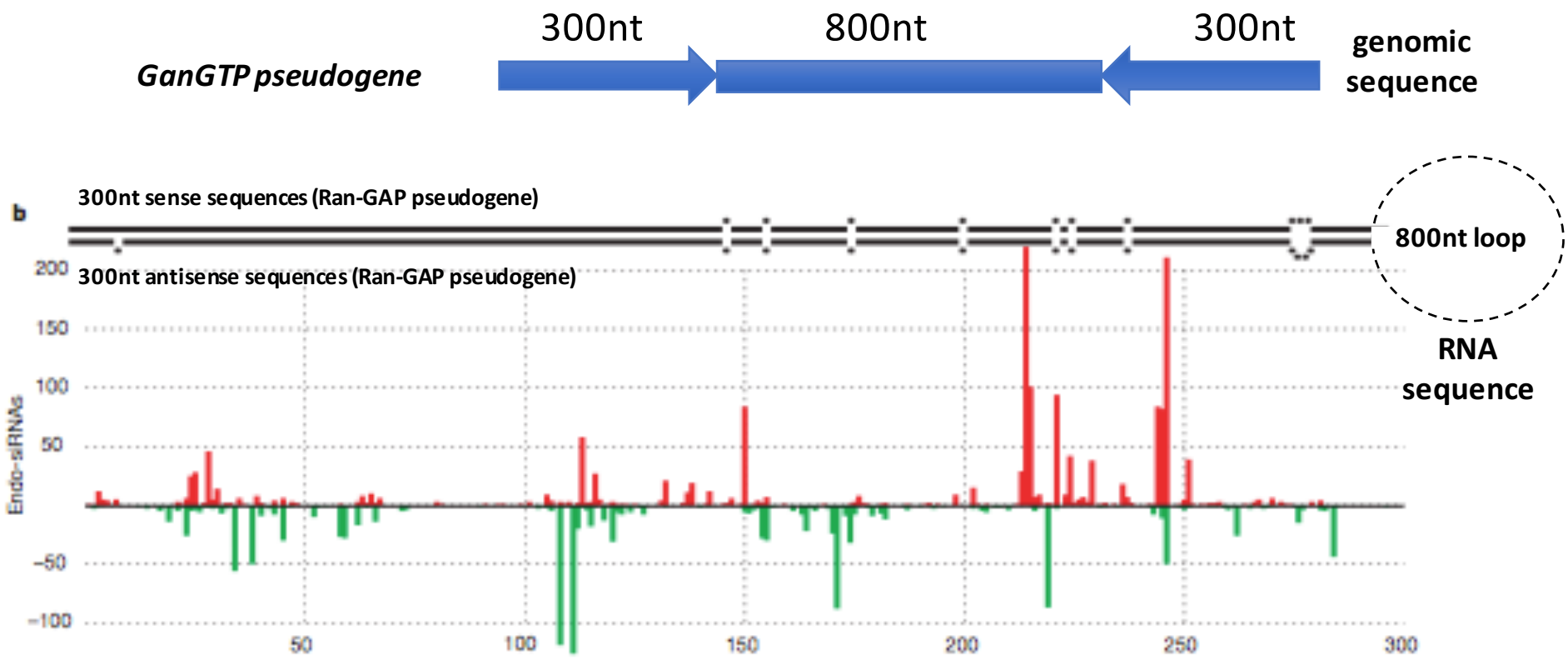
Located at different position in the genome



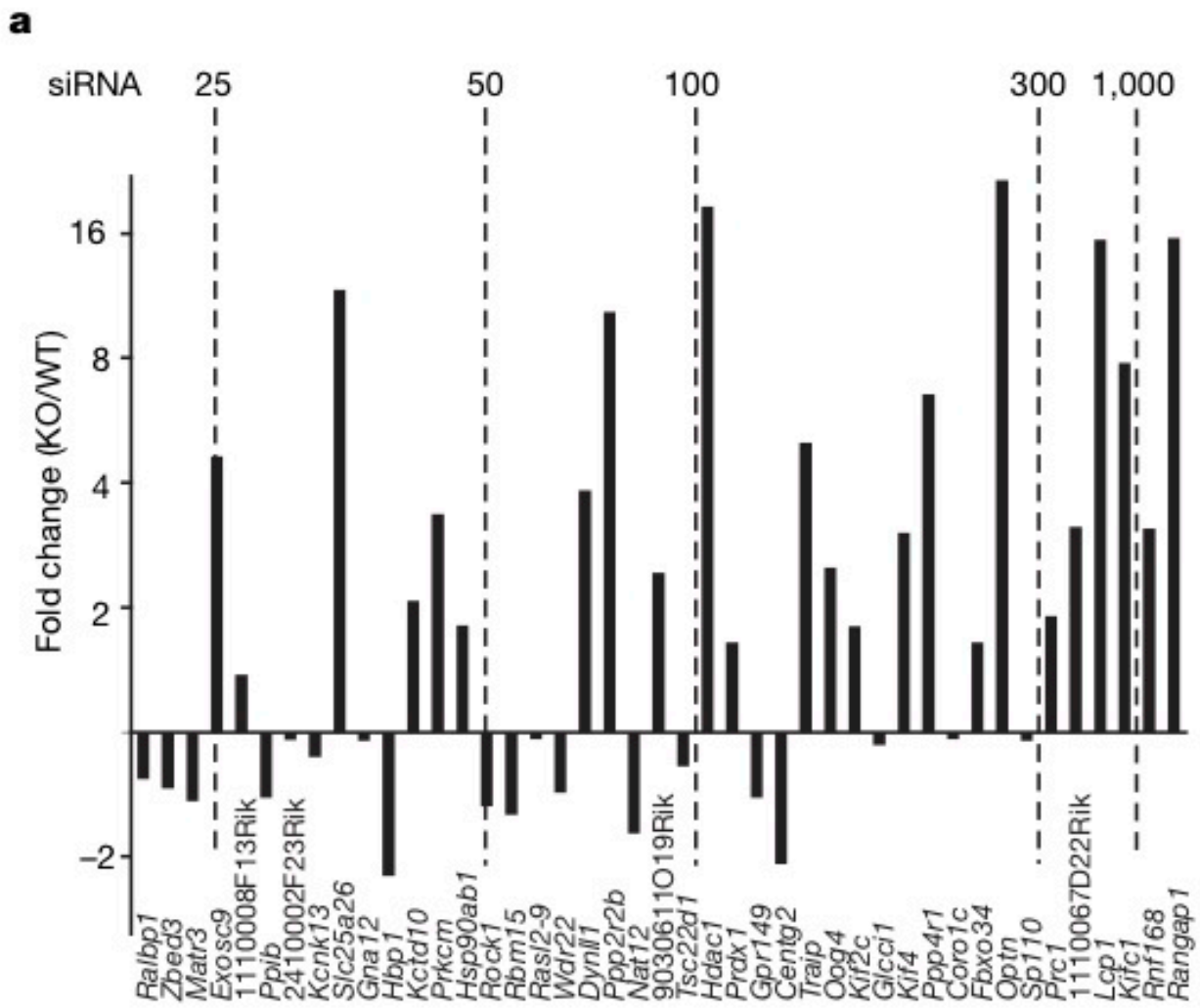
Discovery of pseudogene derives endo-siRNAs

PseudogeneGTPase-activating protein for Ran (Ran-GAP)

Pseudogene contains a 300 bp inverted repeat and and intervening 800 bp loop siRNAs can be detected on regions were RNAs from inverted repeats overlap.



A large set of endo-siRNAs are involved in gene regulation



How to elegantly demonstrate the action of siRNAs

Take small RNA sequencing data

→ list of siRNA sequences

→ Match to annotated mRNAs

→ Select candidate genes expressed in ES cells

Use Dicer wt and Dicer $^{-/-}$ Embryonic stem cells

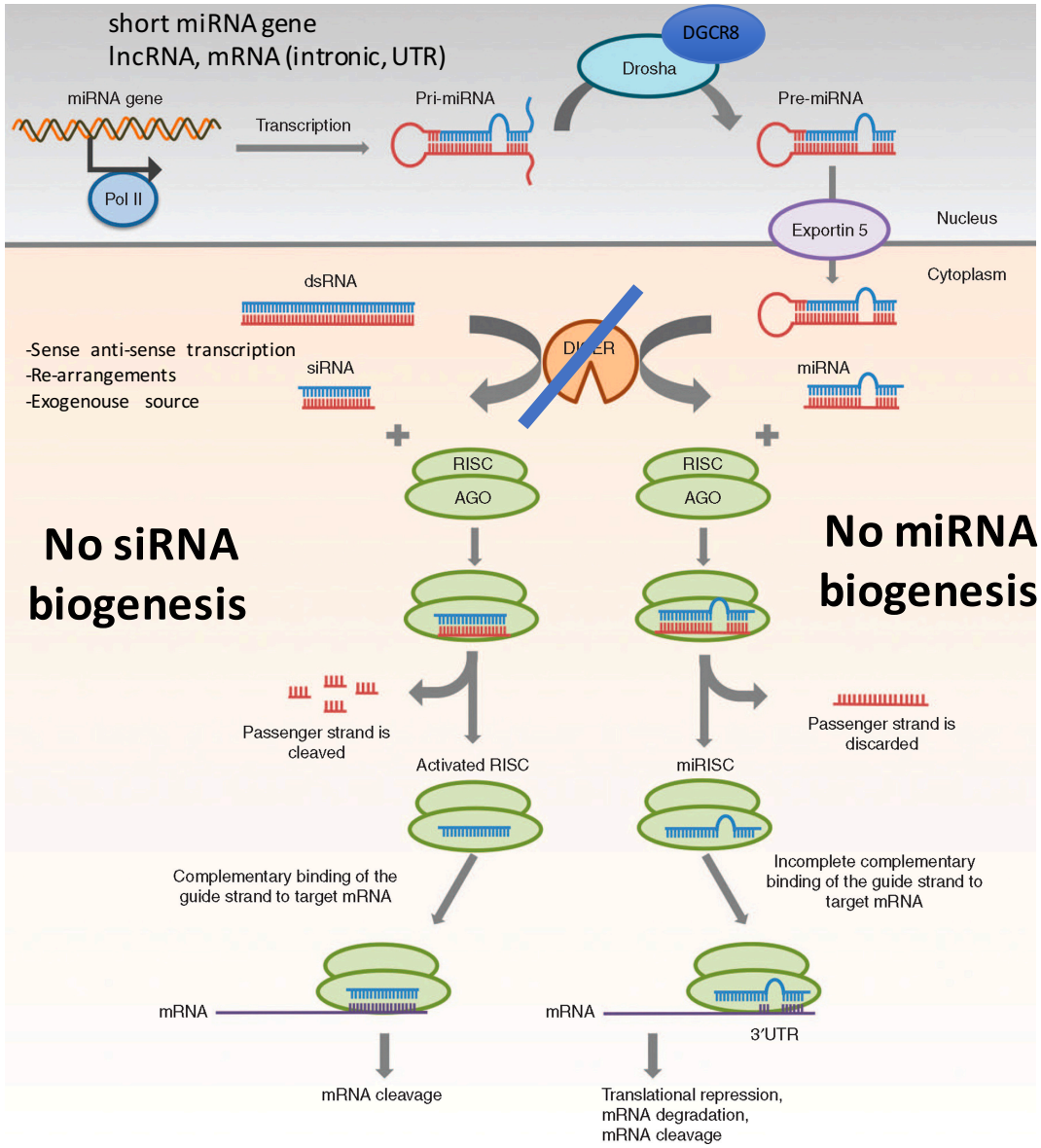
- Prepare total RNA

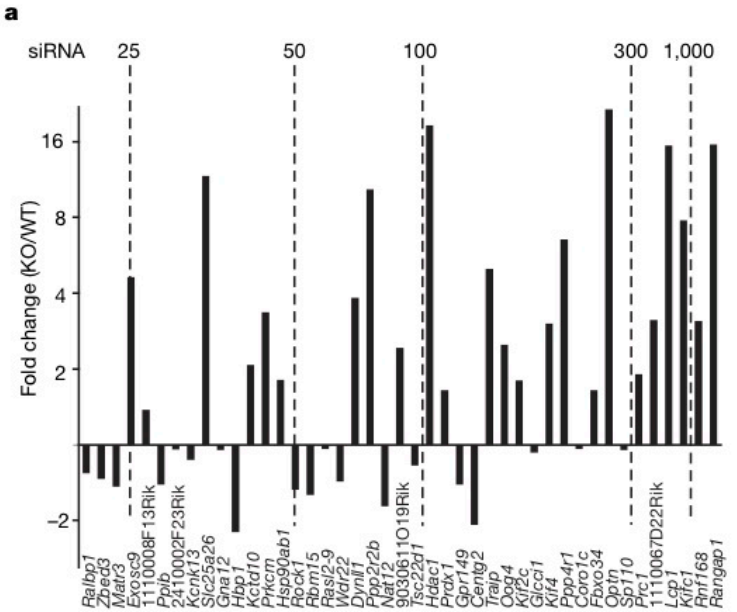
- Make reverse transcription

-Measure the expression of genes with overlapping siRNAs

A large proportion of genes associated with endo-siRNAs is upregulated in Dicer null ES cells

siRNA and miRNA biogenesis and gene regulation





No Dicer

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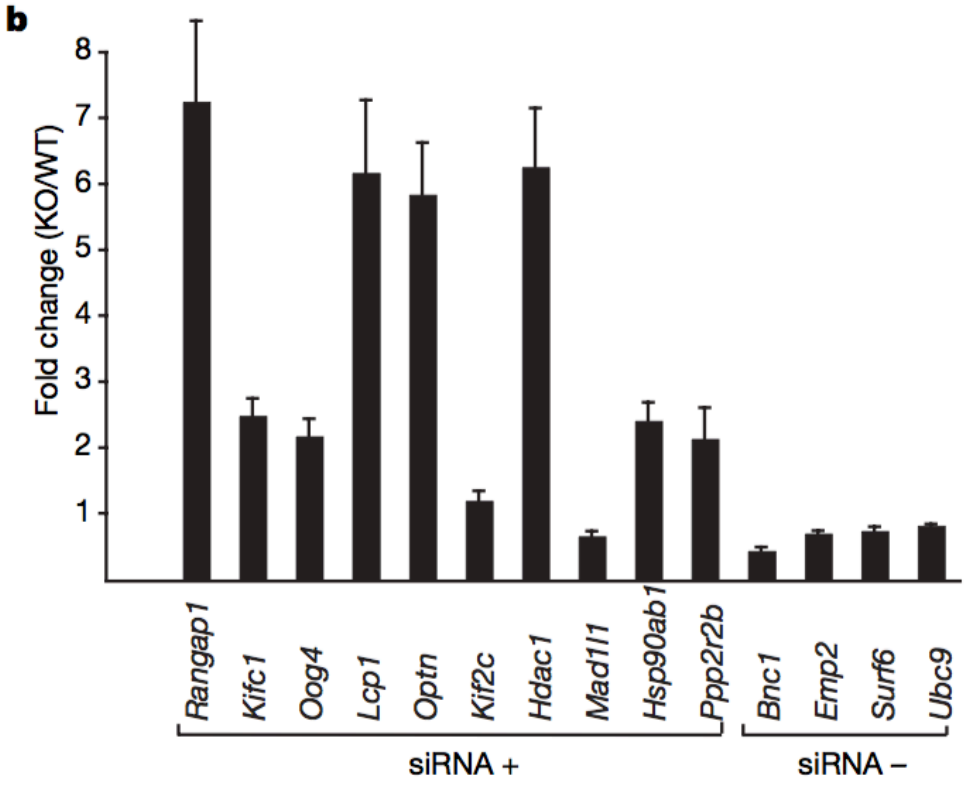
No siRNA biogenesis

↓

siRNA targets upregulated

A large set of endo-siRNAs are involved in gene regulation



Genes with corresponding siRNA reads found in sequencing data

Higher expression in Dicer-/- versus Dicer wt ES cells

Genes with no corresponding siRNA reads found in sequencing data

Same expression in Dicer-/- versus Dicer wt ES cells

Embryonic stem cells (murine)

Dicer wild type
Dicer $^{-/-}$

- Prepare total RNA

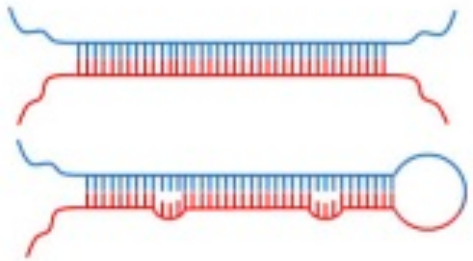
- Make reverse transcription

-Measure the expression of genes with overlapping siRNAs (siRNA +) or without Overlapping siRNAs (siRNA-)

Generation of endo-siRNAs

Human/mouse

Endogenous dsRNA precursors



dsRNA, long hairpins
sense/antisense hybrids

Ago-1
Ago-2
Ago-4?

Post –transcriptional repression
Transposon control
Chromatin modification

A source for anti-sense transcripts:
-Antisense pseudogenes
-Transcribed inverted repeats
-NATs: naturally ocurring antisense transcripts
-Frequently also antisense transcripts of transposable elements

sense and antisense transcripts can base-pair and form dsRNA

↓

Processing by Dicer

↓

siRNA formation

↓

Target RNA slicing

Endo-siRNA levels are low in vertebrate species:
no siRNA amplification loop because no RNA dependent Polymerase present!!!
Higher relevance in biological Situations where endo-siRNAs reach higher levels:
→Control of transposable elements
→DNA damage associated expression of small RNAs
→Biological situations
→Associated with the upregulation of sense – antisense forming transcripts