

Lecture I-5_2018

Non-coding long and small RNAs in epigenetic control

- Definition of small RNA classes and their function.
- Basic mechanisms of RNA-interference: enzymes and reactions.
- Concept of transcriptional and posttranscriptional control.
- RNAi mediated control of chromatin/DNA-modifications
- The role of small RNAs in development and disease.

Molecular interaction levels in epigenetics

4 DNA-methylation

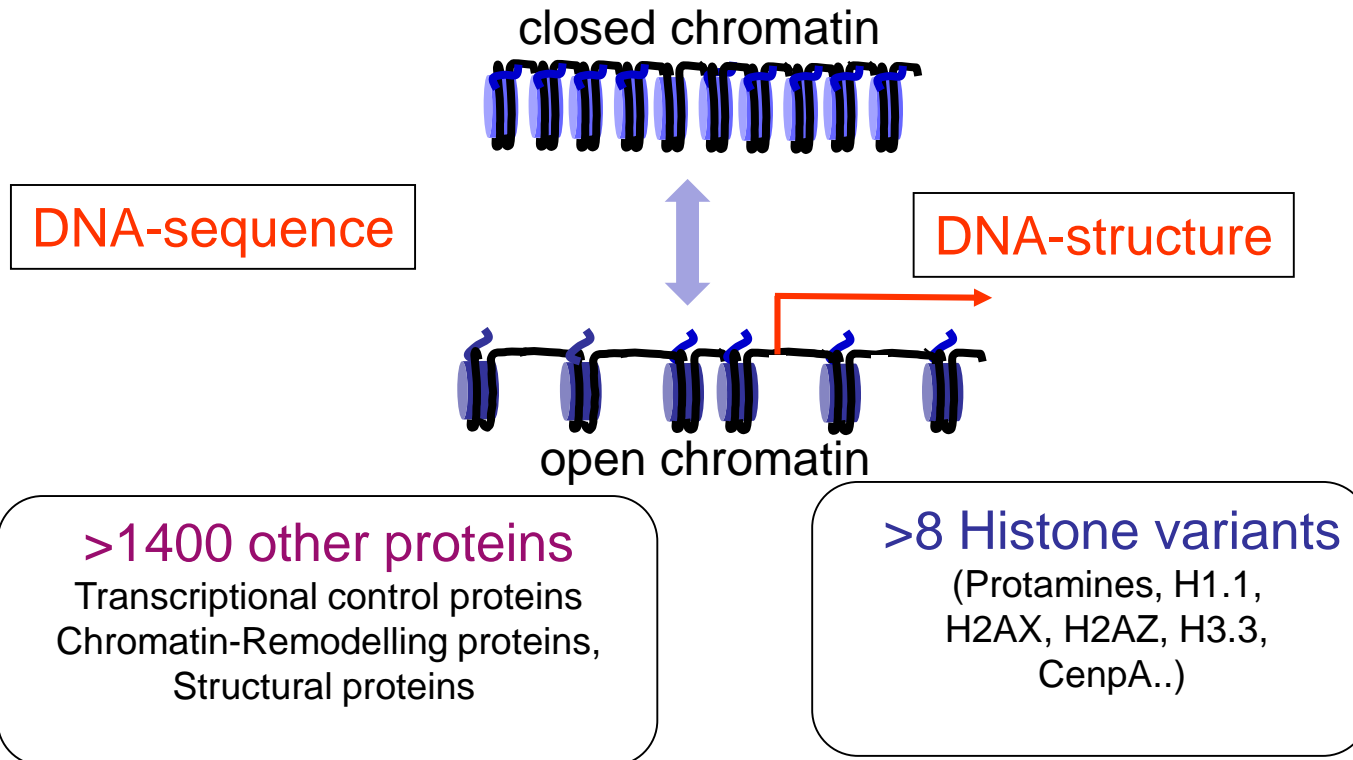
(5^{me}cytosine, 5^{hme}cytosine,
5^{carboxyl}cytosine)

> 10 classes of RNAs

(mi-, pi-, si- and
long non-coding RNAs)

>140 Histone Mod's

(Methylation, Acetylation,
Phosphorylation,...)



Non-coding RNAs – regulatory function

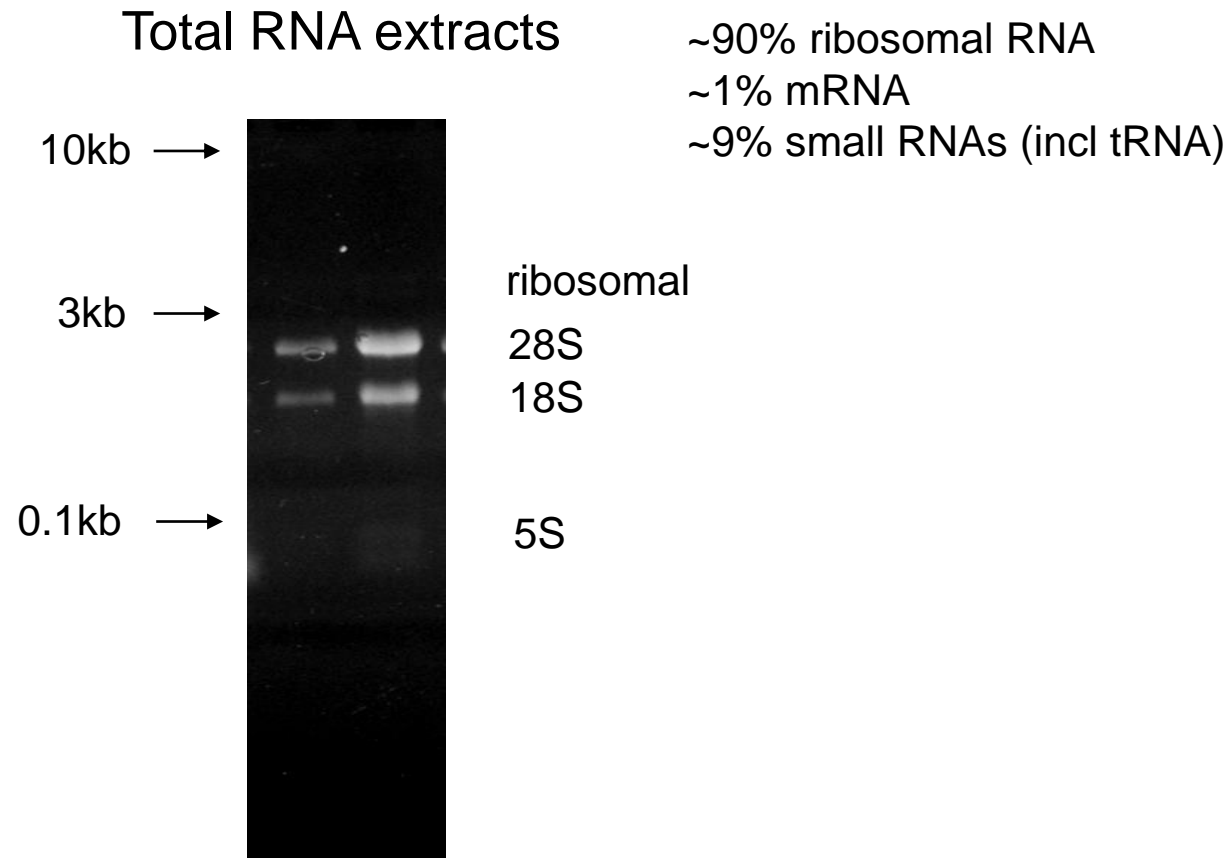
RNA classes

- Long non-coding RNAs (>200bp)
- Small non-coding RNAs (20 - 200 bp)

Mechanisms

- Post transcriptional gene silencing
- miRNA and piRNA biogenesis
- Transcriptional Silencing
- Post transcriptional Silencing

RNA abundance

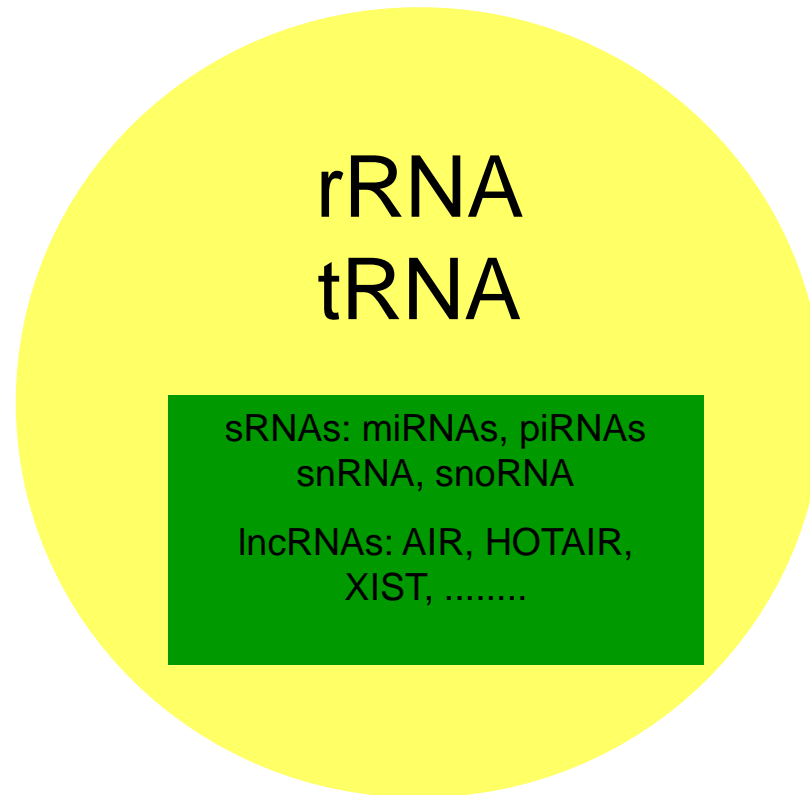


RNA abundance

Coding RNA



Non coding RNA



Functional RNA
Involved in
Translation,
Splicing, Modification

Regualtory RNA
affecting Gene
expression

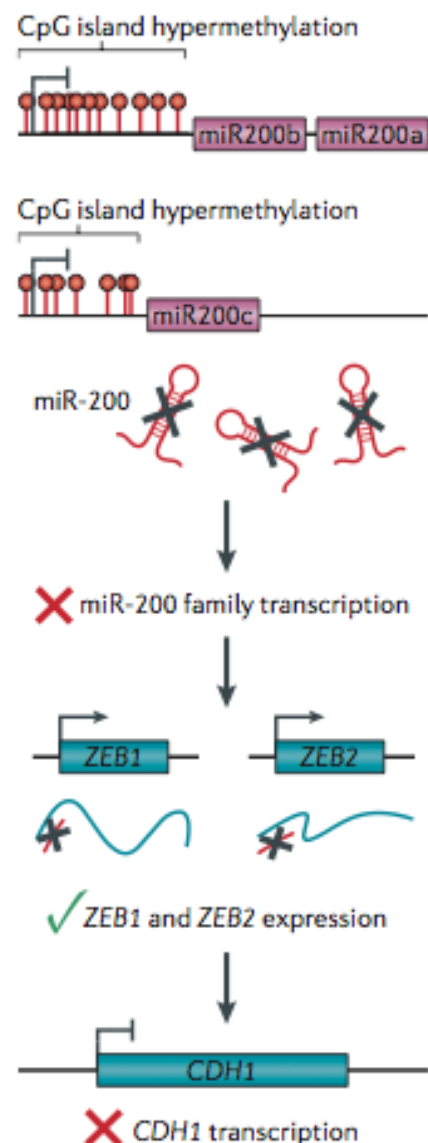
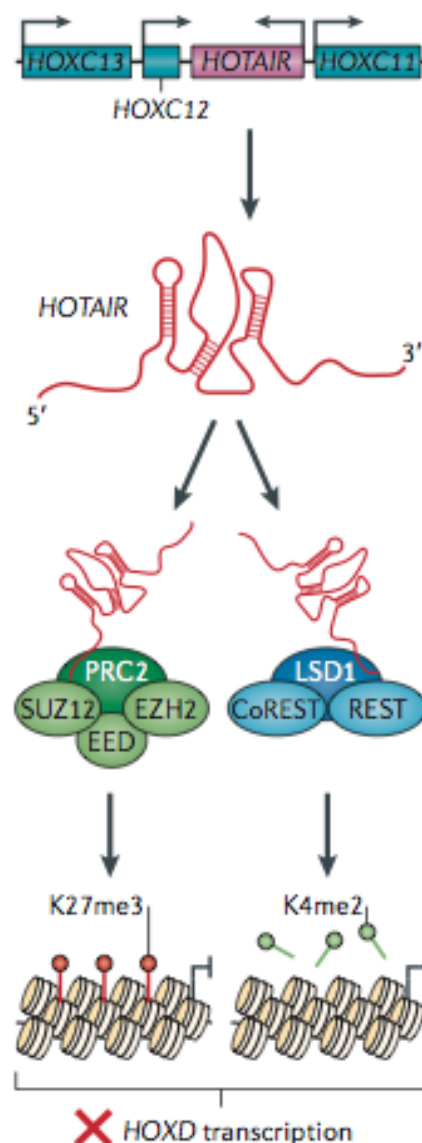
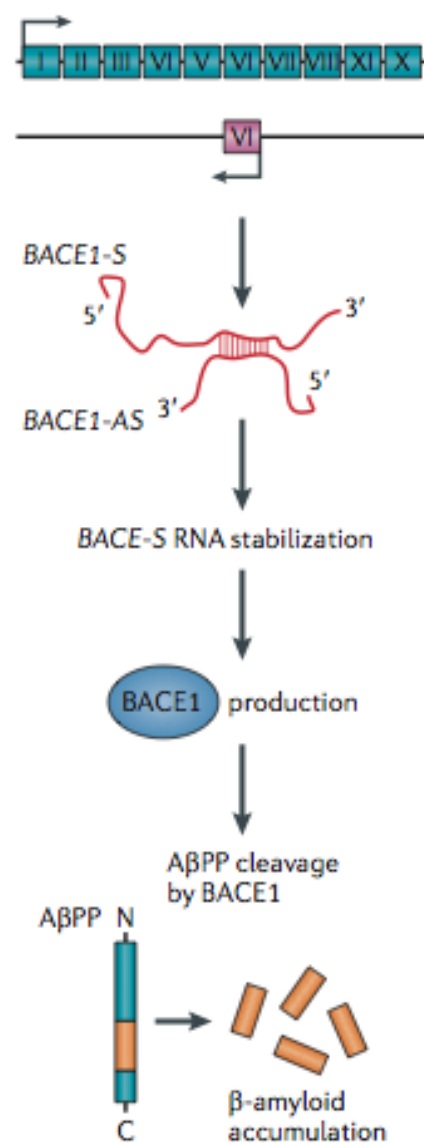
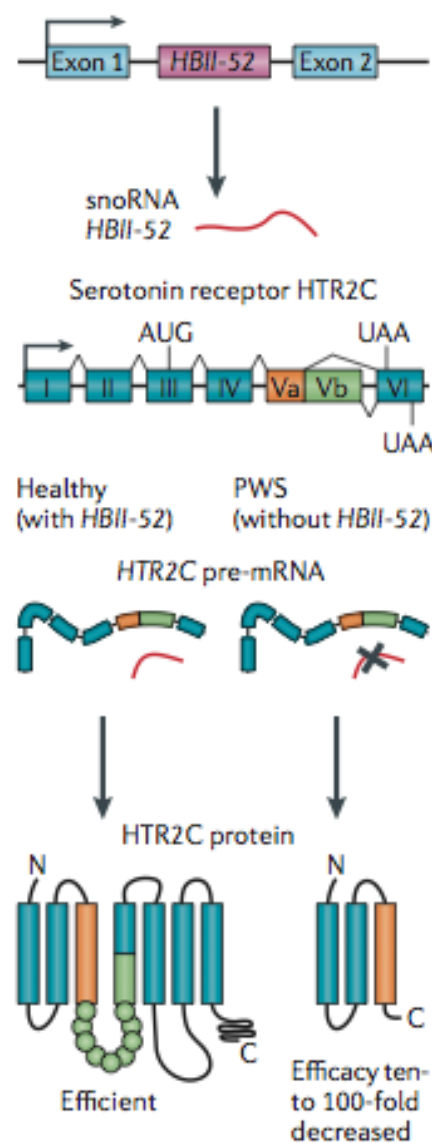
a miR-200 in cancer**b lincRNA HOTAIR****c lncRNA in Alzheimer's disease****d snoRNA HBII-52 in PWS**

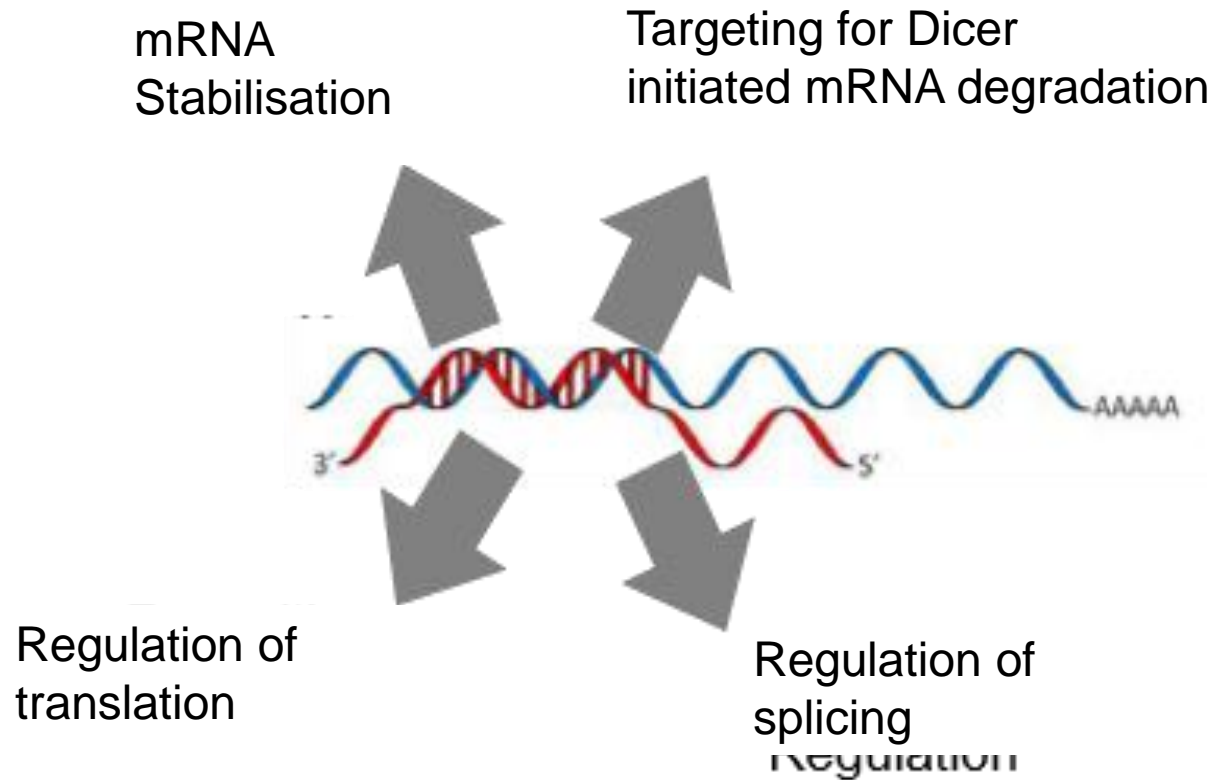
Figure 2 | **Examples of roles of ncRNAs in disease pathophysiology.** a | miR-200 is an example of a microRNA

Figure 2 | Examples of roles of ncRNAs in disease pathophysiology. **a** | miR-200 is an example of a microRNA (miRNA) whose role in cancer is well characterized. Alterations in the epigenetic regulation of the miR-200 family are involved in epithelial-to-mesenchymal transition (EMT) in cancer. Specifically, CpG island hypermethylation-associated silencing of these miRNAs in human tumours causes an upregulation of the zinc finger E-box-binding homeobox (HOX) 1 (ZEB1) and ZEB2 transcriptional repressors, which, in turn, leads to a downregulation of E-cadherin (CDH1) — these are changes that promote EMT. **b** | The lincRNA HOX transcript antisense RNA (*HOTAIR*) is involved in polycomb retargeting across the genome. *HOTAIR* expression is increased in transformed cells and induces a genome-wide promoter re-occupancy by polycomb- and H3K27-trimethylation-associated silencing of target genes, such as the HOX protein *HOXD*. The effect of these changes is to increase cancer invasiveness. **c** | lncRNA targeting of β -secretase 1 (BACE1) has a role in the pathophysiology of Alzheimer's disease. An antisense lncRNA, *BACE1-AS*, regulates the expression of the sense *BACE1* gene (labelled *BACE1-S* in the figure) through the stabilization of its mRNA. *BACE1-AS* is elevated in Alzheimer's disease, increasing the amount of BACE1 protein and, subsequently, the production of β -amyloid peptide. **d** | The role of the snoRNA in Prader–Willi syndrome (PWS). The loss of the snoRNA in PWS changes the alternative splicing of the serotonin receptor *HTR2C* precursor mRNA (pre-mRNA), resulting in a protein with reduced function. A β PP, amyloid- β precursor protein; CoREST, REST corepressor.

lncRNA

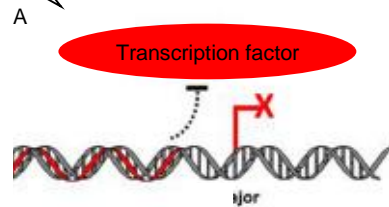
- lncRNA size: >200 nucleotides
- Transcribed by Pol II: carry 5' -Cap and Poly A-Tail
- lncRNAs show differential expression
- Current estimation: eukaryotic genomes have more lncRNAs than mRNA producing loci!!!
- lncRNAs have separate regulation but are often antisense transcripts of coding genes

Antisense lncRNAs can act post-transcriptionally

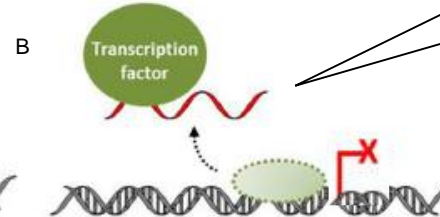


lncRNAs acting transcriptionally

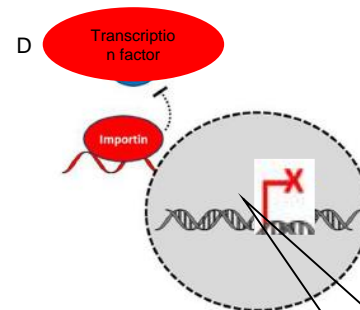
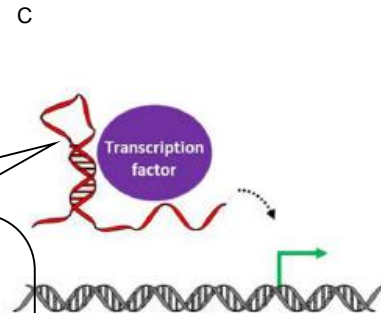
Binding of the promoter DNA:
Triplex Structure inhibiting
binding of transcription factors



lncRNAs can bind to
Transcription factors and
block them.

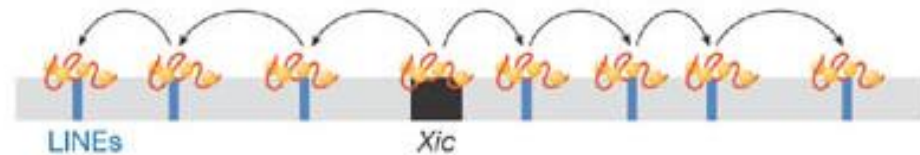
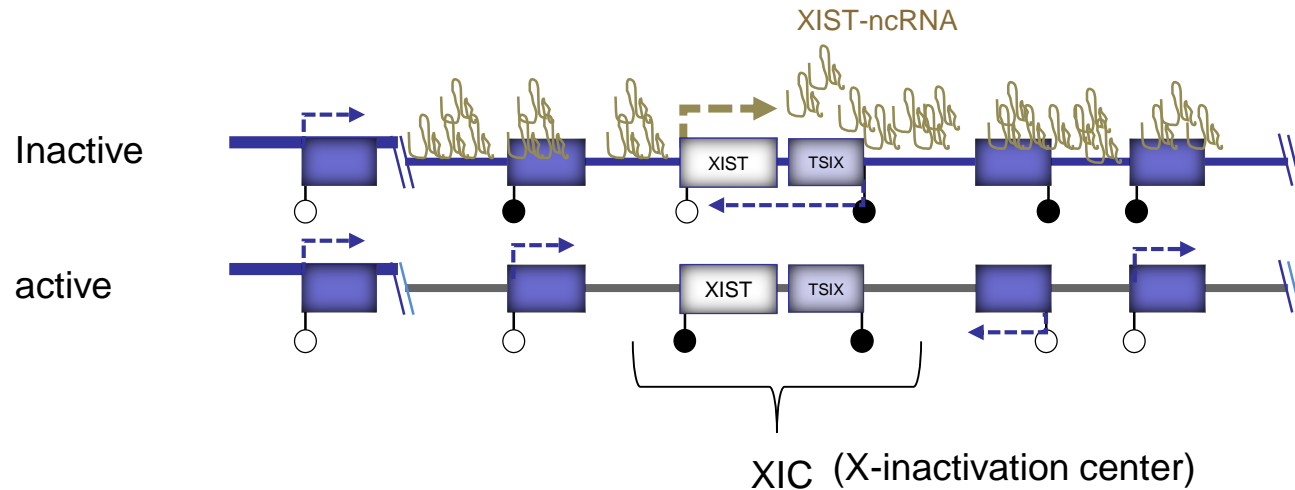


lncRNA can be activated by
binding to transcription factors and
target them to promoters using
RNA-DNA homology.

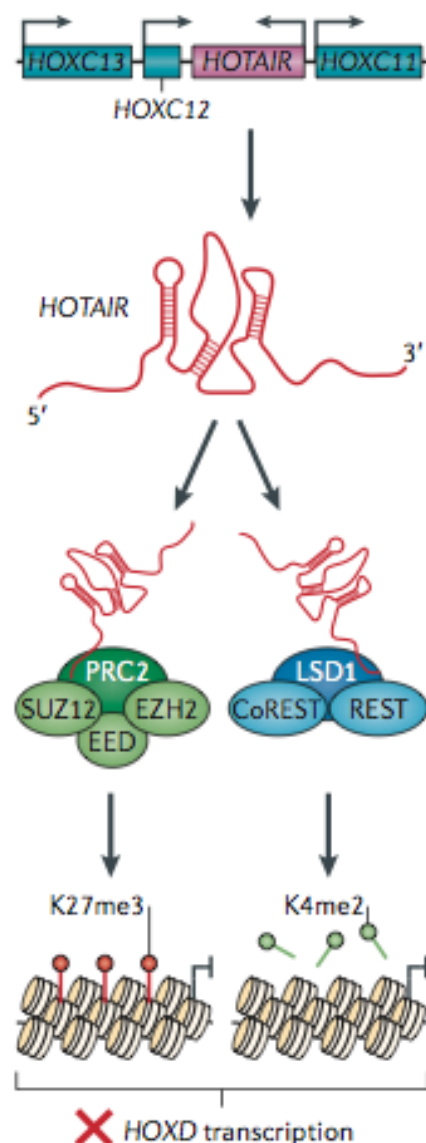


lncRNAs have been
shown to bind to
transcription factors in the
cytosol thus inhibiting
their transport into the
nucleus

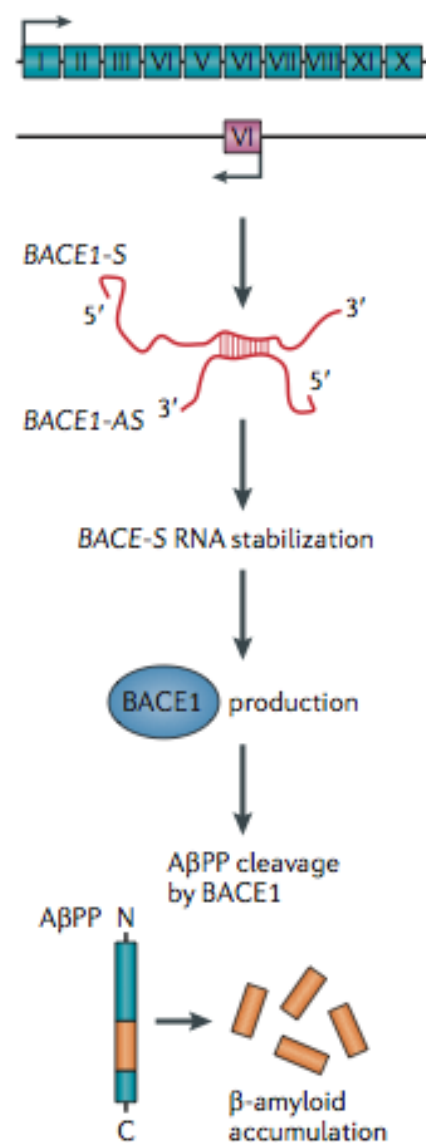
The Xist RNA is essential for the initiation of X-Chromosome silencing in mammals



b lincRNA *HOTAIR*



c lncRNA in Alzheimer's disease



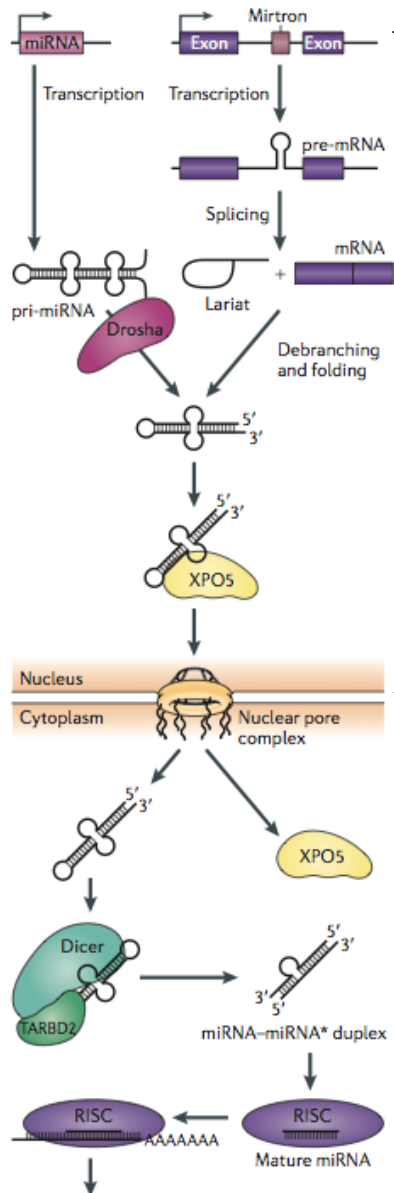
Examples of roles of ncRNAs in disease pathophysiology. a | miR-200c

2. small Regulatorische RNA: siRNA / miRNA

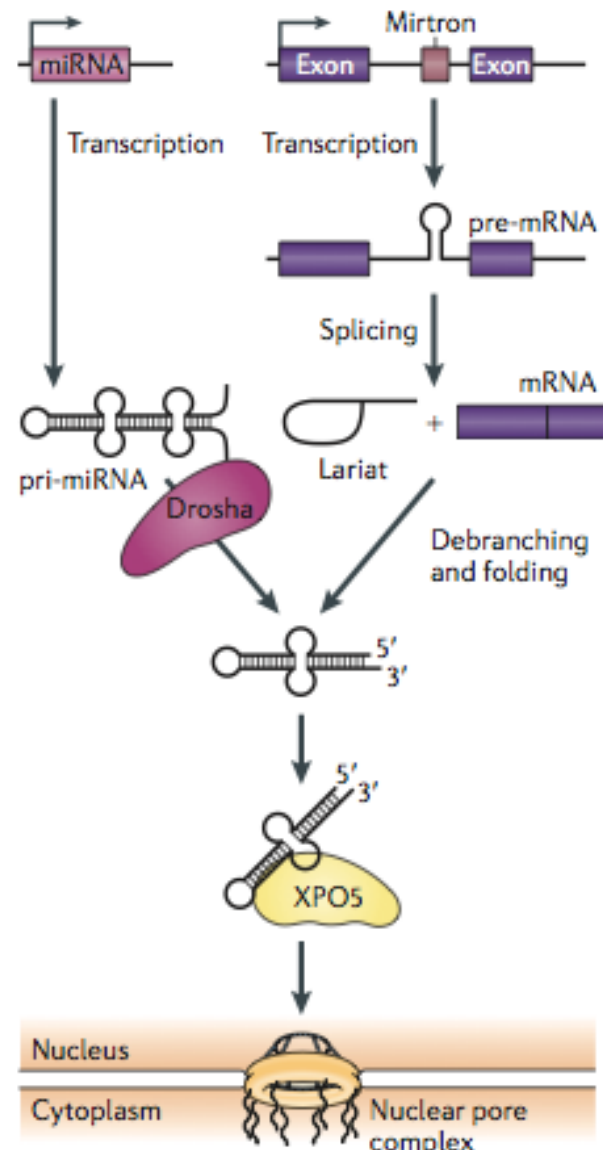
- Length between 20 to 30 nucleotides
- Are formed by processing dsRNAs (e.g. sense-antisense)
- Require Dicer and Argonaut (Ago) (RISC-complex) for their „activity“
- classes of endogenous regulatory small RNAs are:
 - miRNA (micro RNAs) – transcriptional and translational modulation
 - piRNA – repression of transposon expression in germ cells
 - priRNA/siRNA - Establishment of heterochromatin at centromeres

mi-RNA Biogenesis

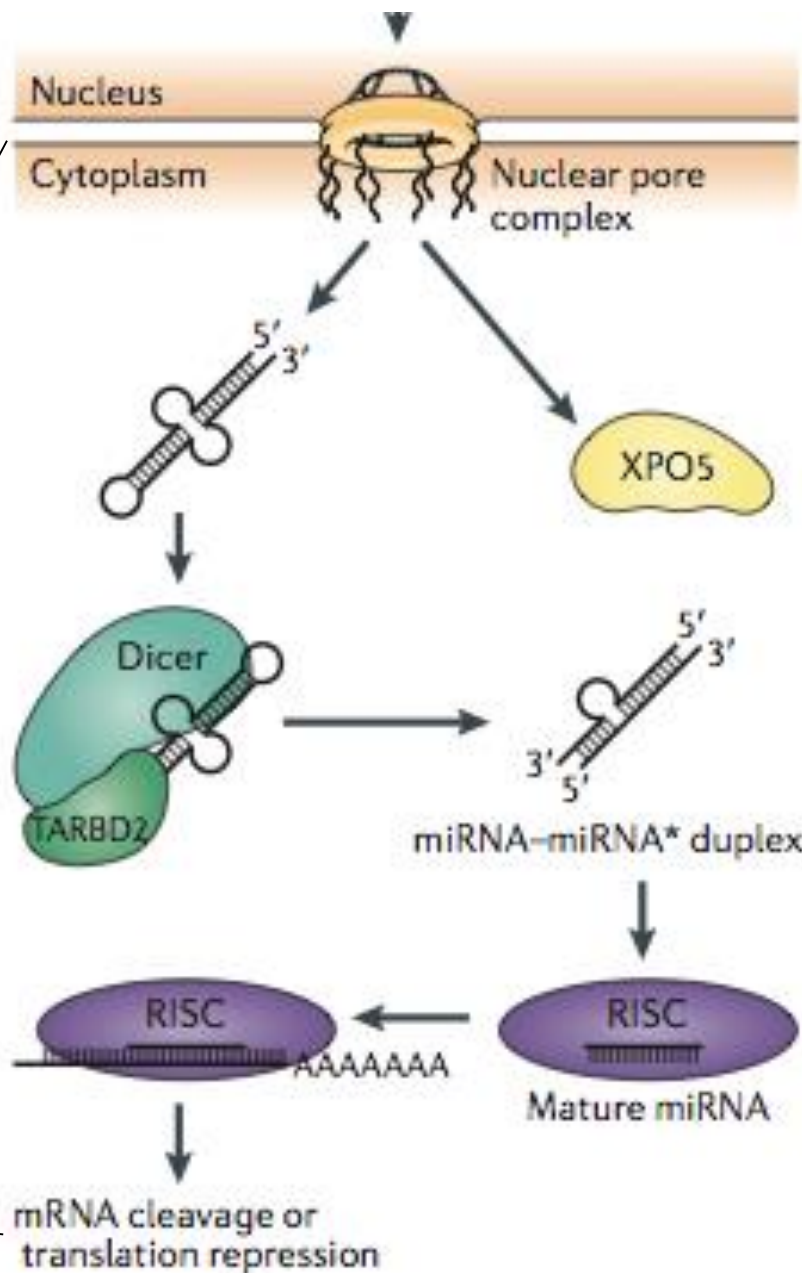
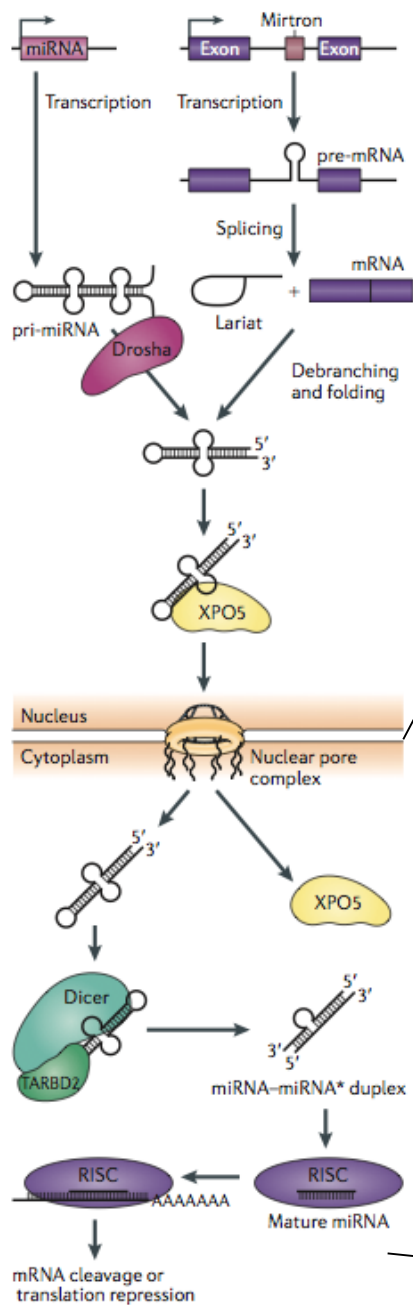
a microRNAs



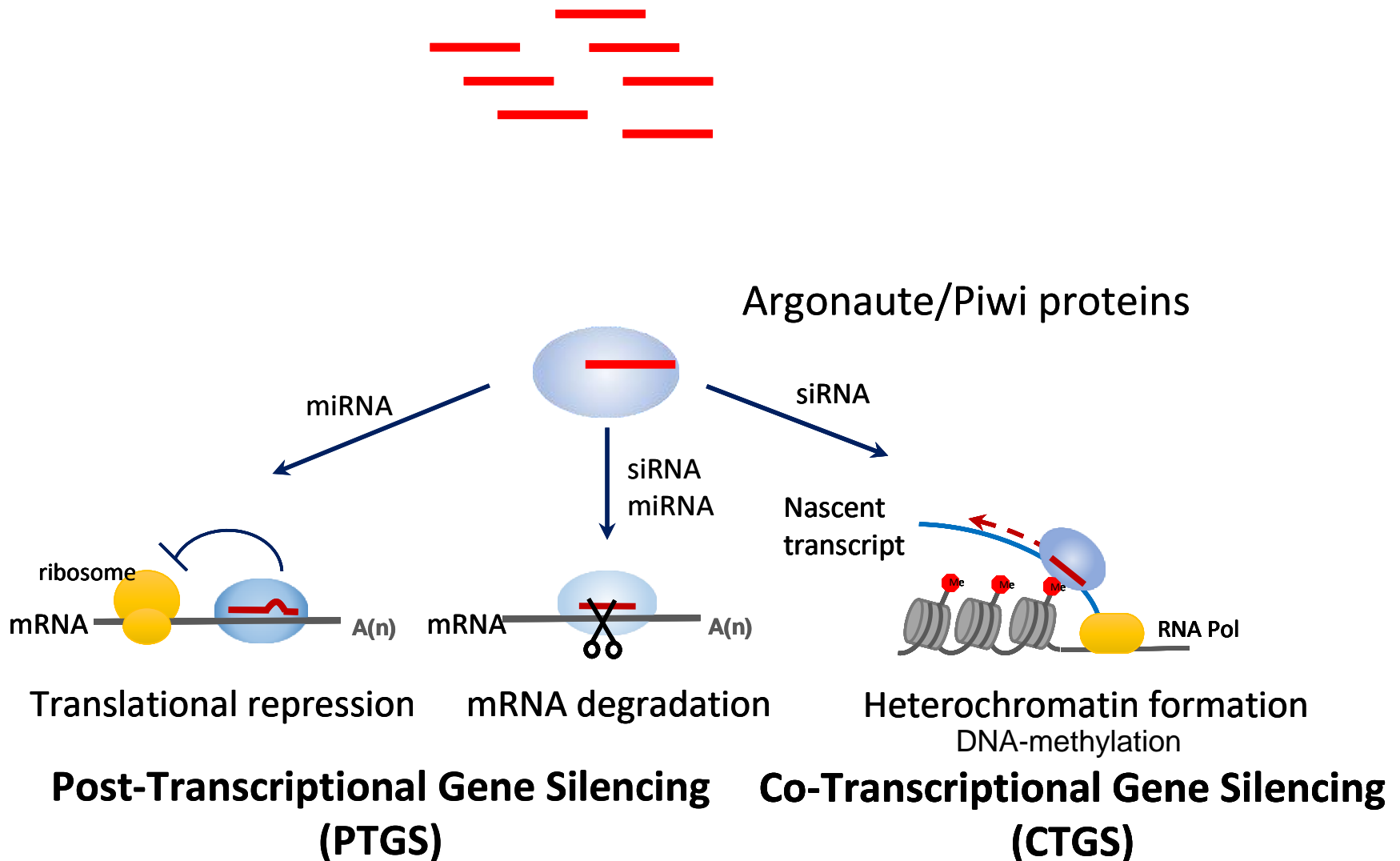
a microRNAs



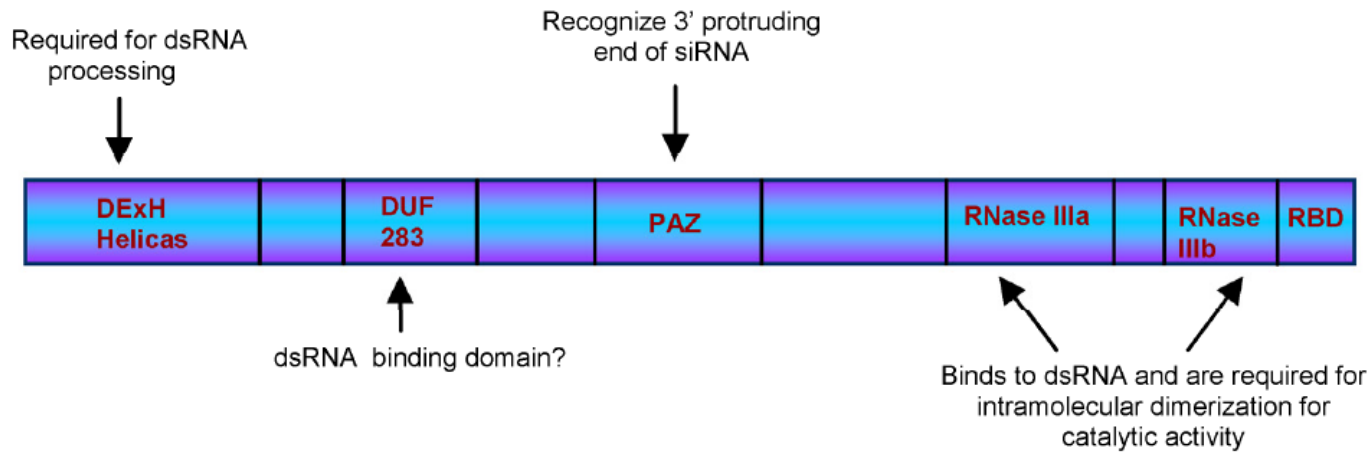
a microRNAs



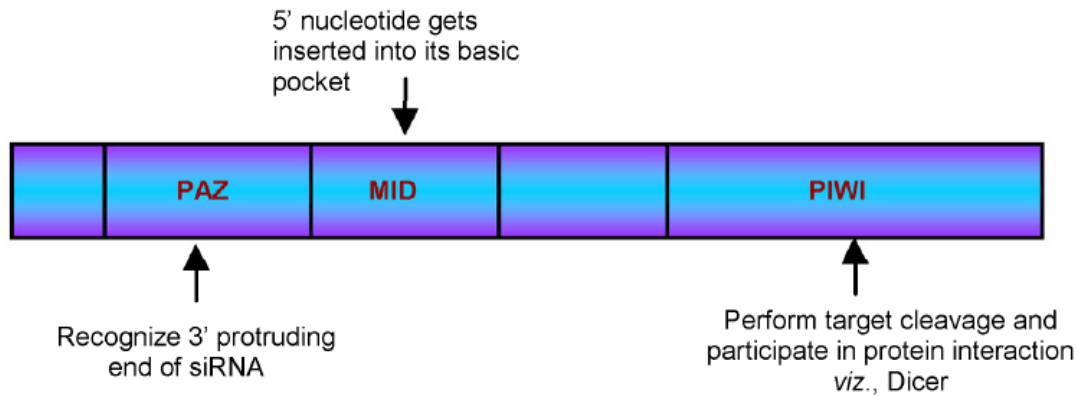
RNAi pathways: many possibilities to knock down gene expression



Dicer



Ago



DUF: Domain of Unknown Function

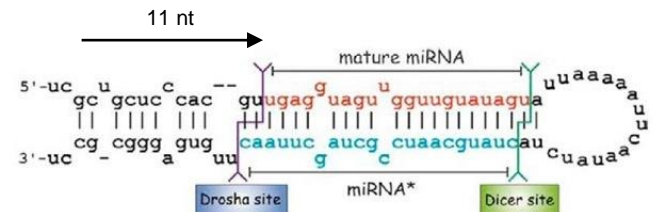
RBD: RNA Binding Domain

PAZ : Piwi, Argonaute and Zwille

PIWI: P element-induced wimpy testes

Characteristics of canonical miRNAs

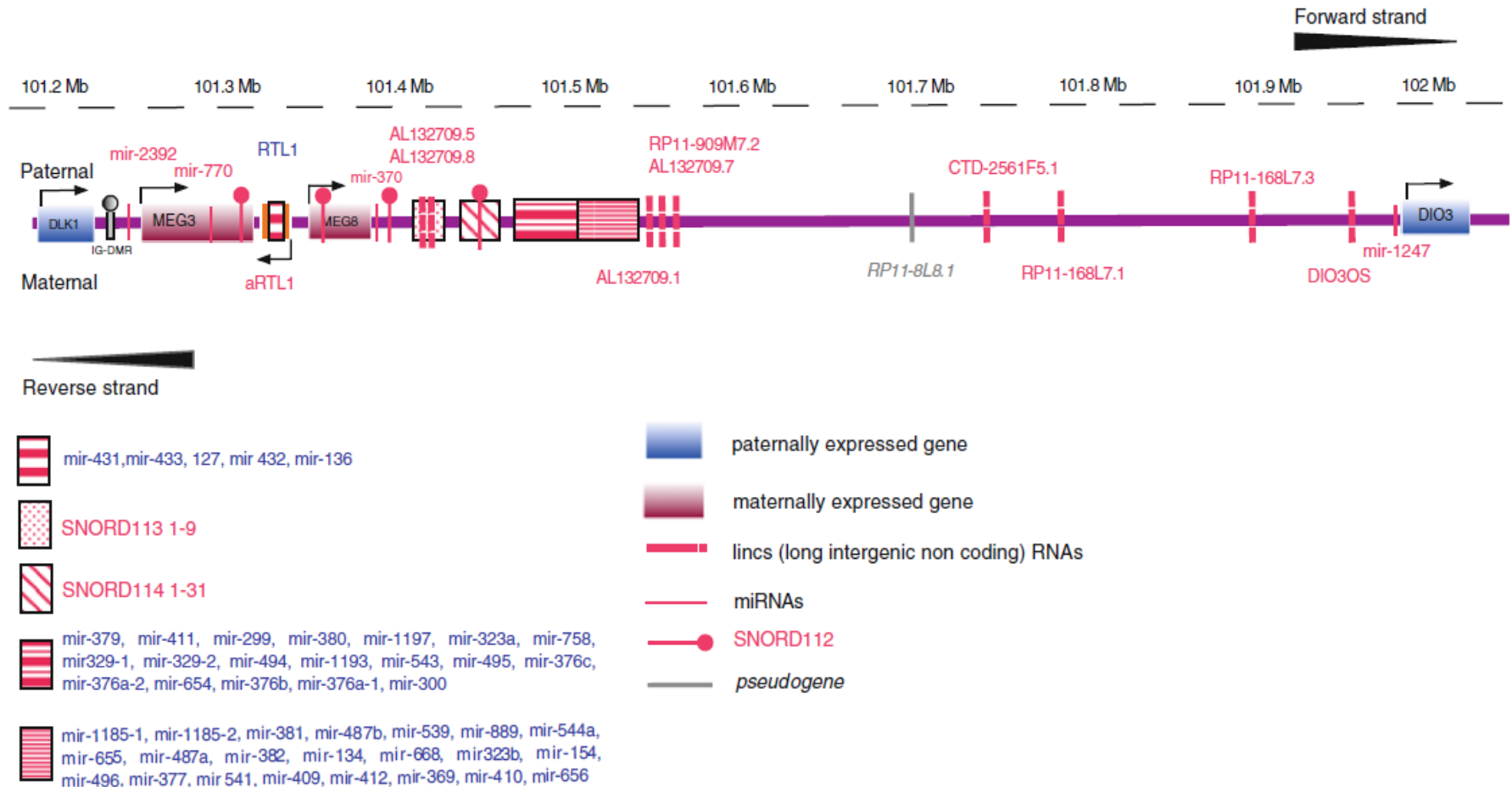
- miRNA gene transcribed by Pol II
- Pri miRNAs are 5' capped and 3' polyadenylated
- Pri miRNAs fold back to imperfect hairpins
- Drosha cuts 11nt from dsRNA end
- Dicer cuts ~22nt away from the Drosha cleavage site.



- miRNAs own the 5'-Seed region. Ca. 7-8nt: this have to bind to the mRNA mRNA only (in plants: 100% of the miRNA must be homolog)
- Most animal miRNAs targeten mRNAs in the 3'-UTR



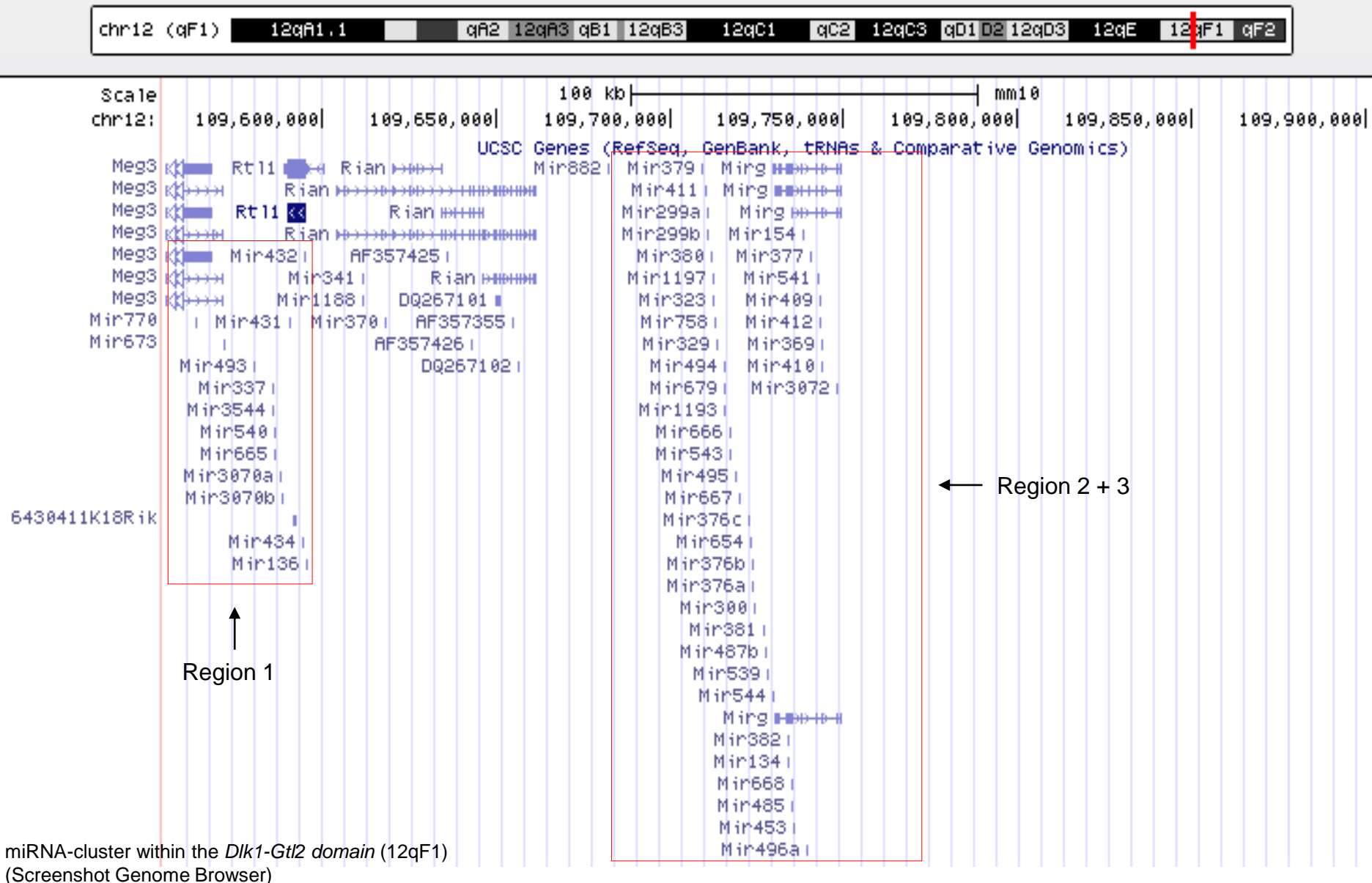
Dlk1-Gtl2 domain of the mouse



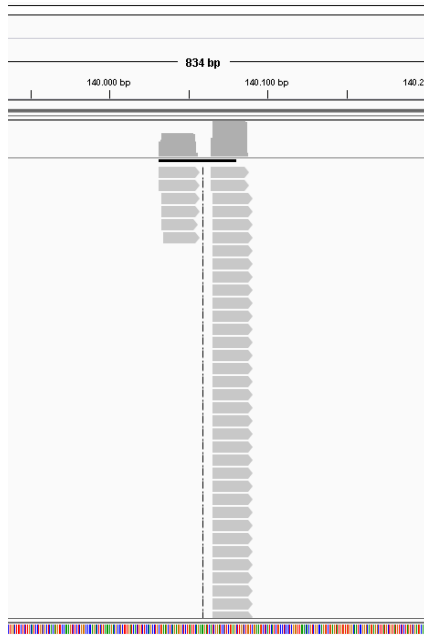
(Benetatos L et al., 2013)

- chromosome 12 (distal domain; 12qF1); ~1Mb
- maternally imprinted region
- Imprinting control center: IG-DMR locus (intergenic, germline-derived- differentially methylated region)
- contains a large number of non-coding RNAs (snoRNAs, ~60microRNAs, lincRNAs)

microRNA CLUSTER WITHIN THE MURINE *DLK1-GTL2* DOMAIN

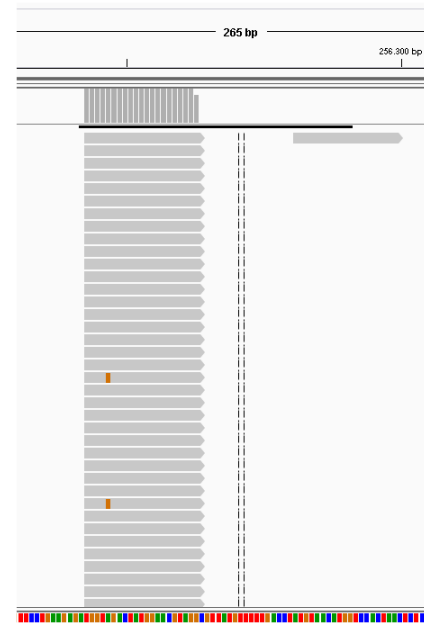


miRNA analysis by sequencing

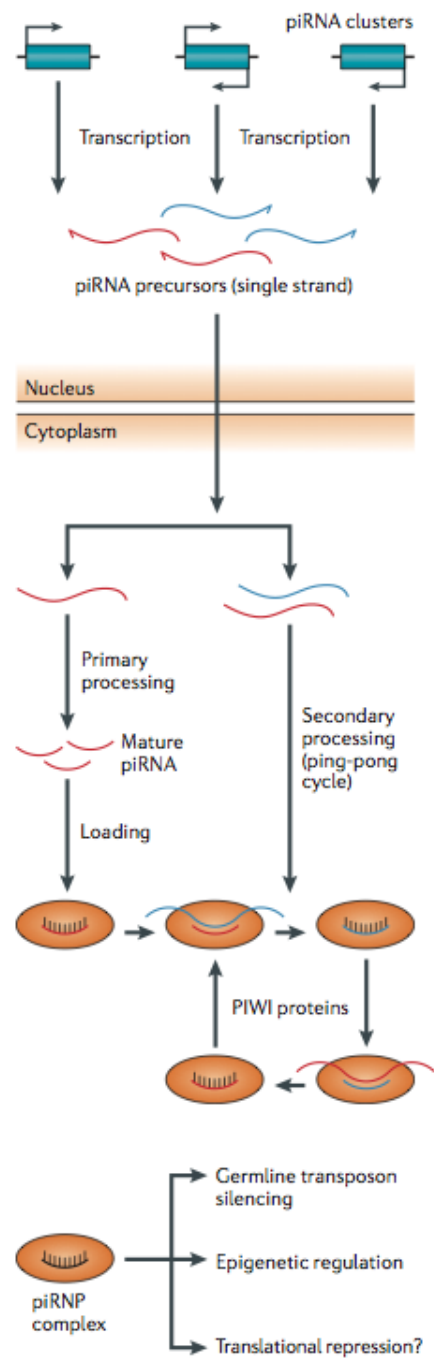
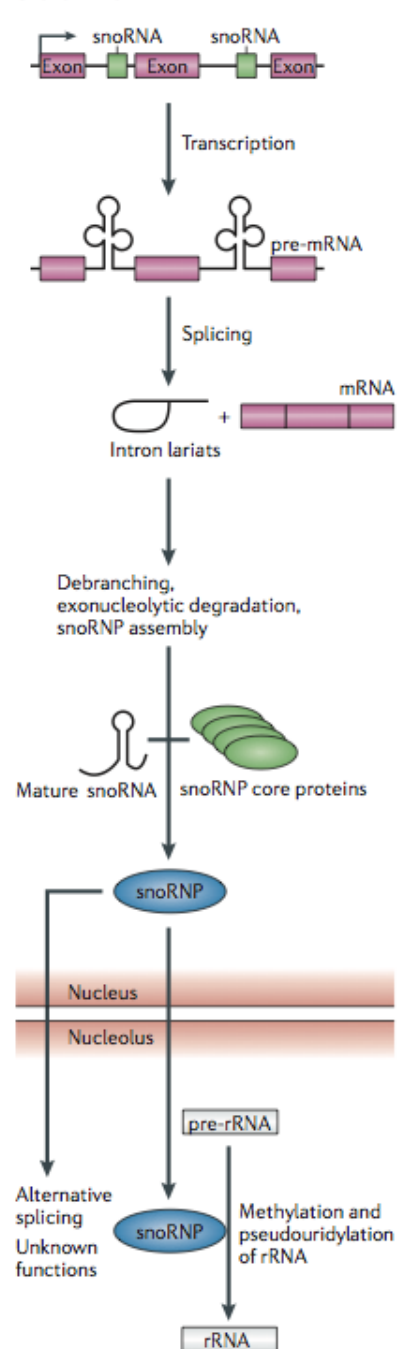


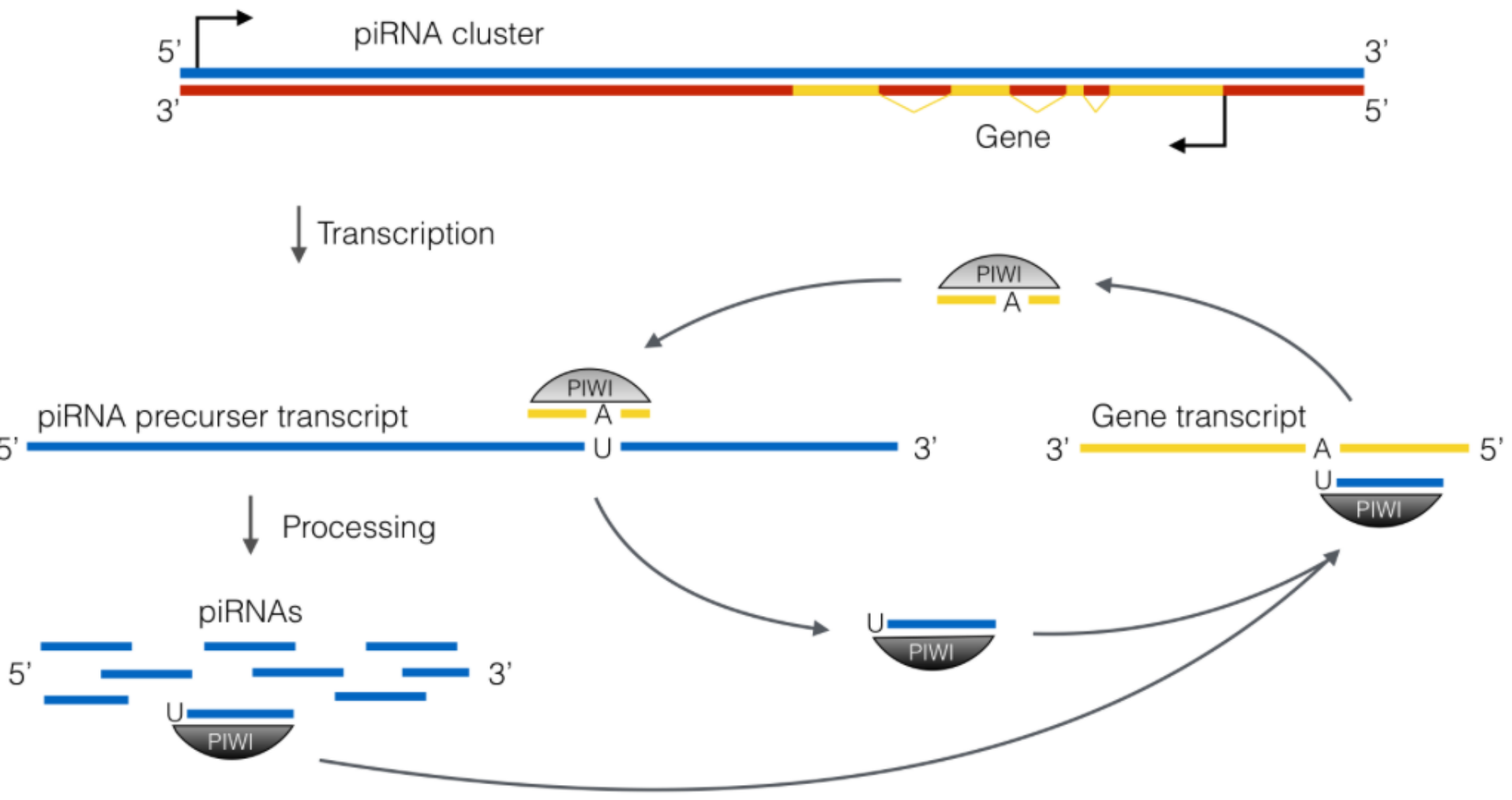
Reads: 776
Ratio 5p/3p:
61/715

Clusters with discrete reads
and predicted structure

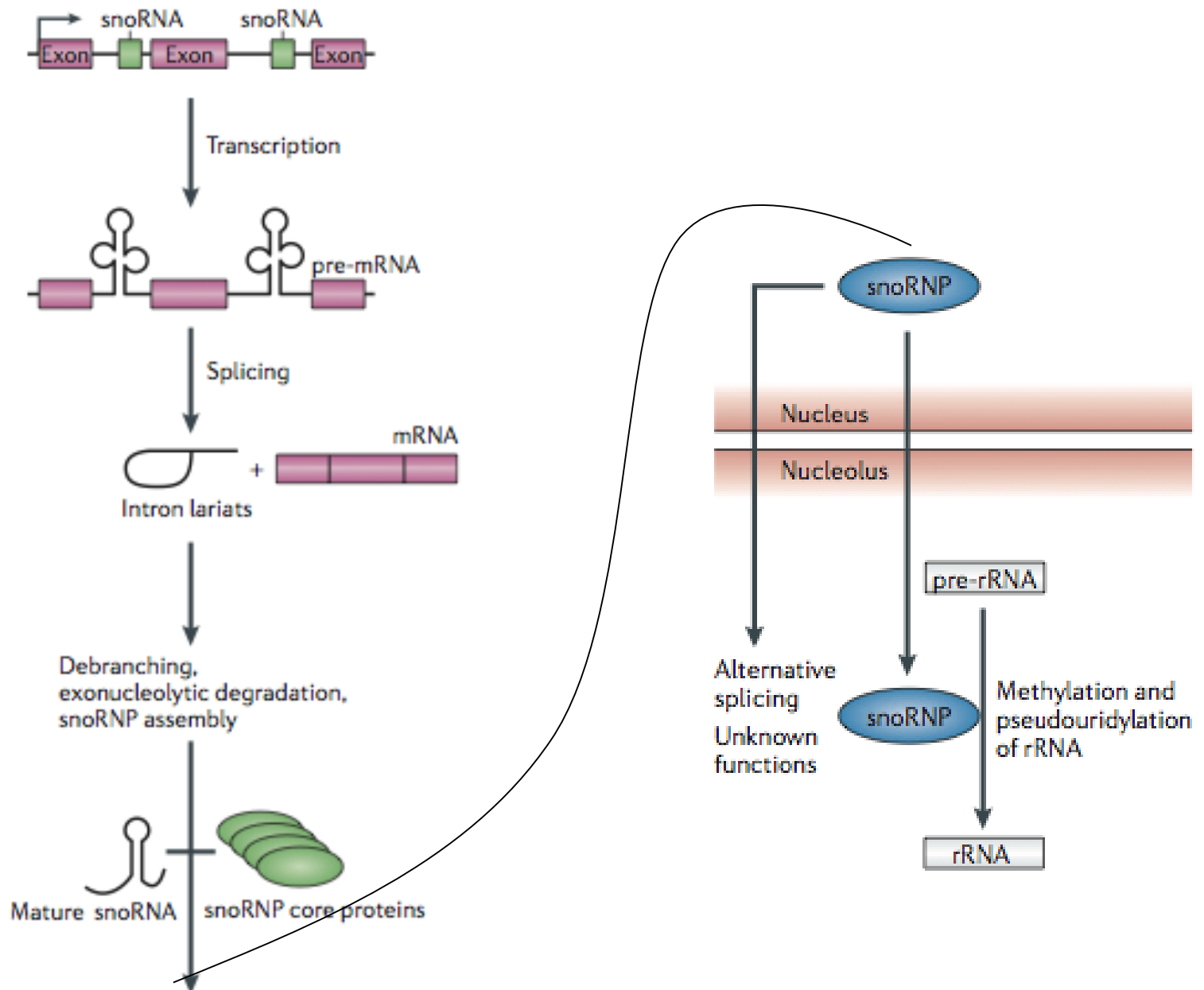


Reads: 675
Ratio 5p/3p:
670/5

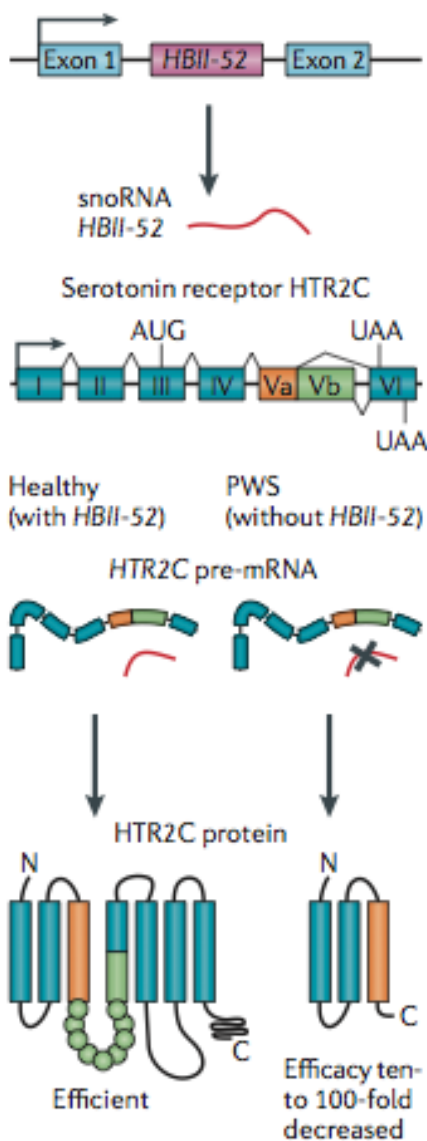
b piRNAs**c snoRNAs**



c snoRNAs

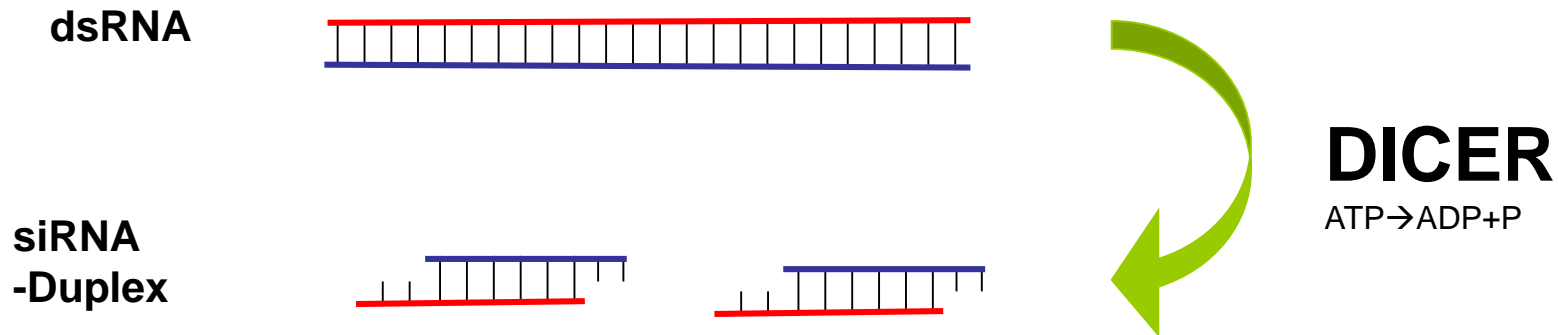


d snoRNA *HBII-52* in PWS



00 is an example of a microRNA

siRNAs are „cleaved“ from dsRNA

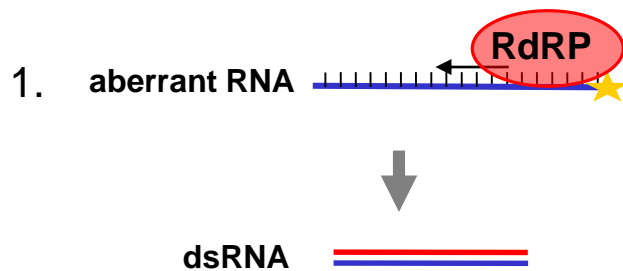


siRNAs often trigger the degradation of the matching mRNA

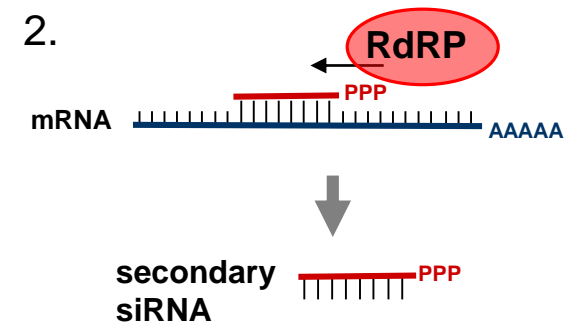
are frequently „used“ (exogenously) for a „knockdown“
(= downregulation) of gene transcription

In Plants siRNAs are produced naturally by RNA dependent RNA Polymerases

RdRP (RNA dependent RNA polymerase)



Synthesis of long dsRNAs from ssRNA template

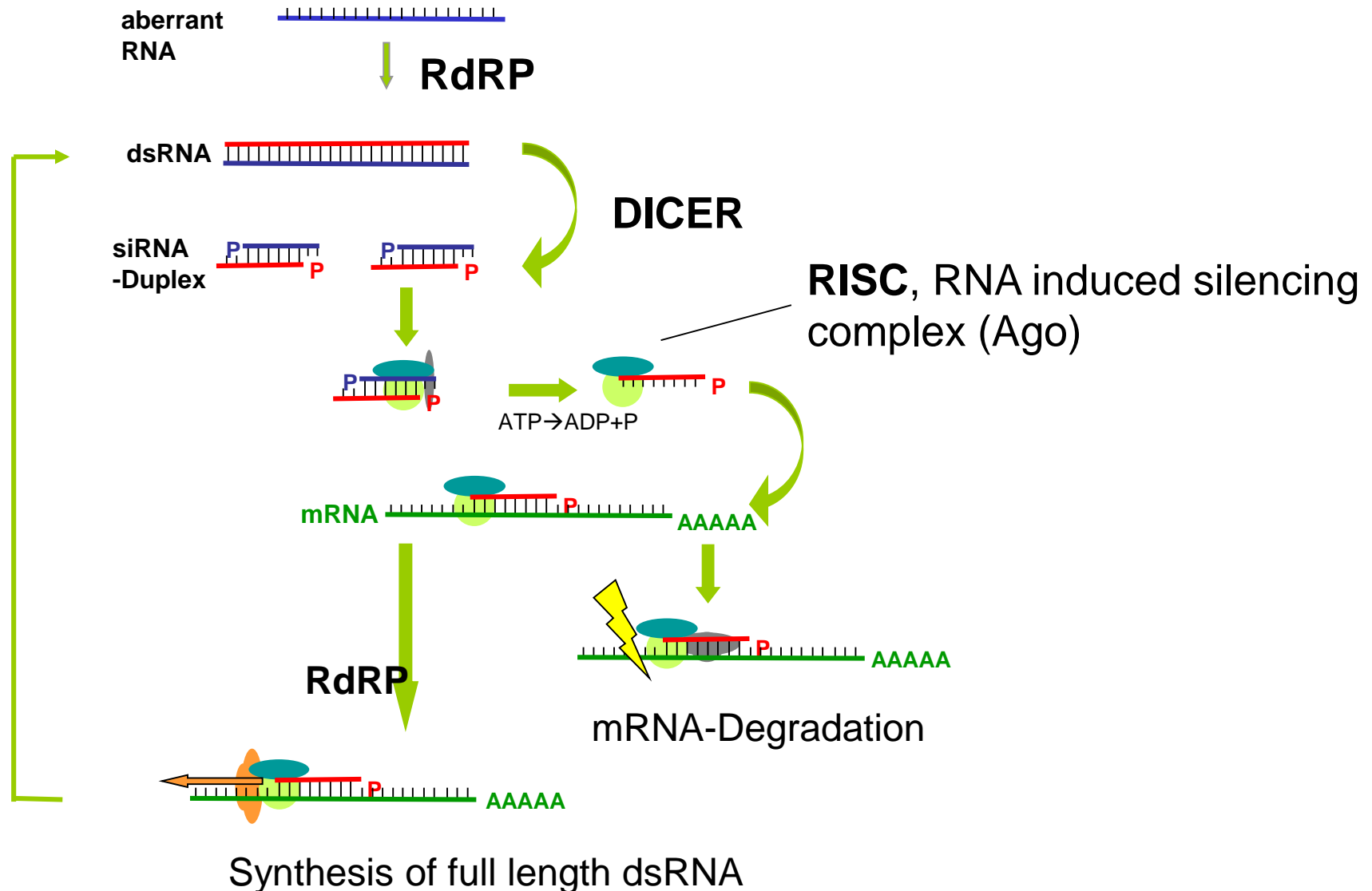


Synthesis of siRNAs from ssRNA template

Eine initial applizierte dsRNA oder siRNA kann zu einen Verstärkungsmechanismus führen bei denen RdRPs sekundäre siRNAs bilden.

Dies kann durch die Synthese eine weiteren langen dsRNA und durch nachfolgen Dicer Schnitt geschehen oder durch die direkte Synthese von sek. siRNAs durch die RdRP.

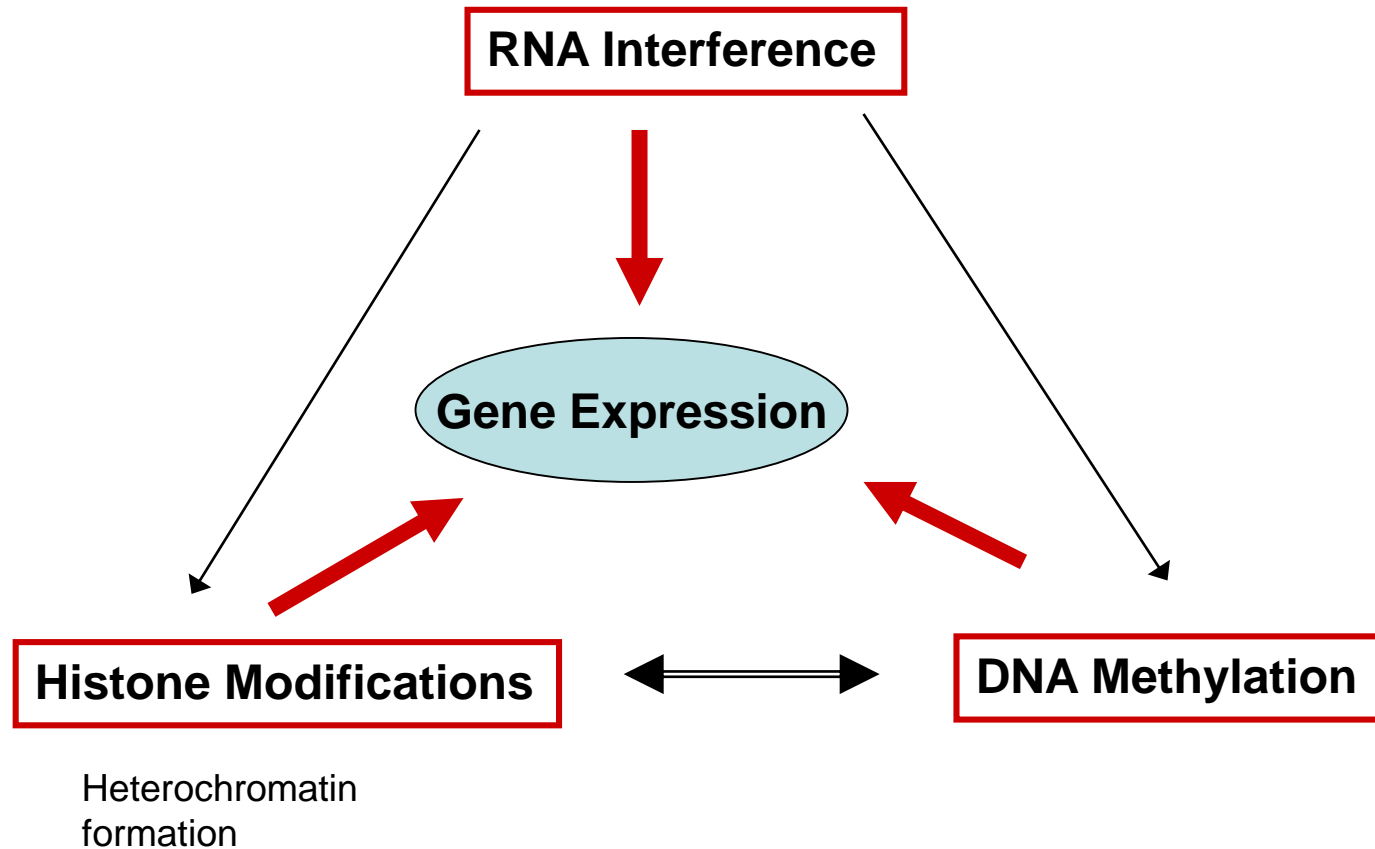
Secondary siRNA synthesis in Plants (*Arabidopsis*) by RdRP



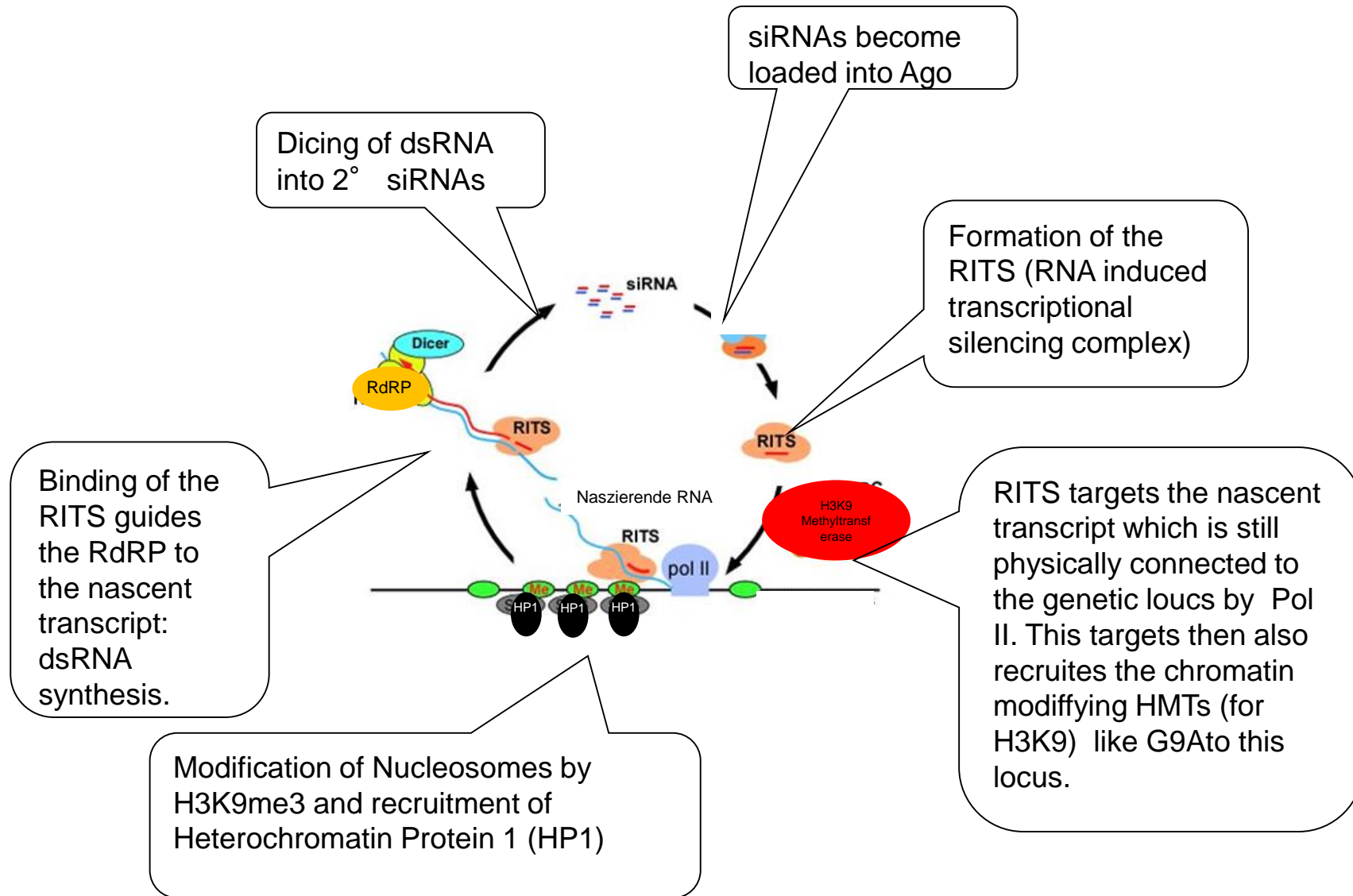
Systemic RNAi in plants

- Spreading of the silencing signal (siRNAs) between cells by plasmodesmata
- The silencing signal seems to follow the phloem flow
- The plant architecture influences the movement of the signal
- Efficient RNA based immune system against virus infections

ncRNAs can directly trigger Epigenetic Mechanisms



Model for Co-transcriptional heterochromatic gene Silencing



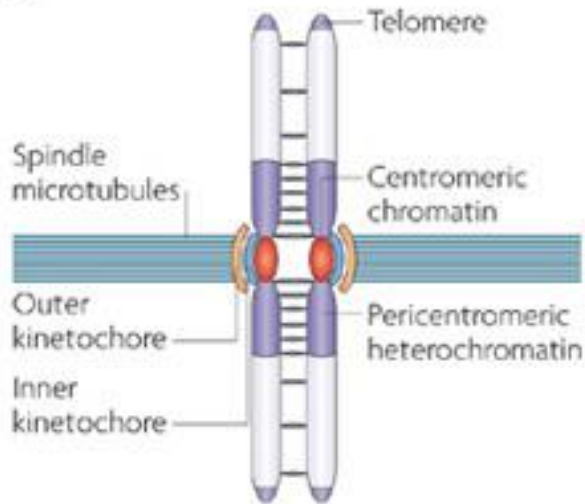
Examples for small RNA mediated heterochromatin formation

- Centromeric heterochromatin in yeast
- Paramutation as an example for Co-transcriptional silencing of protein coding genes

RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast

Ira M. Hall^{*†}, Ken-ichi Noma[†], and Shiv I. S. Grewal^{*††}

a



Centromeric heterochromatin serves as a physical locus where spindle microtubules attach

Segregation of chromatids during late anaphase

C

DAPI

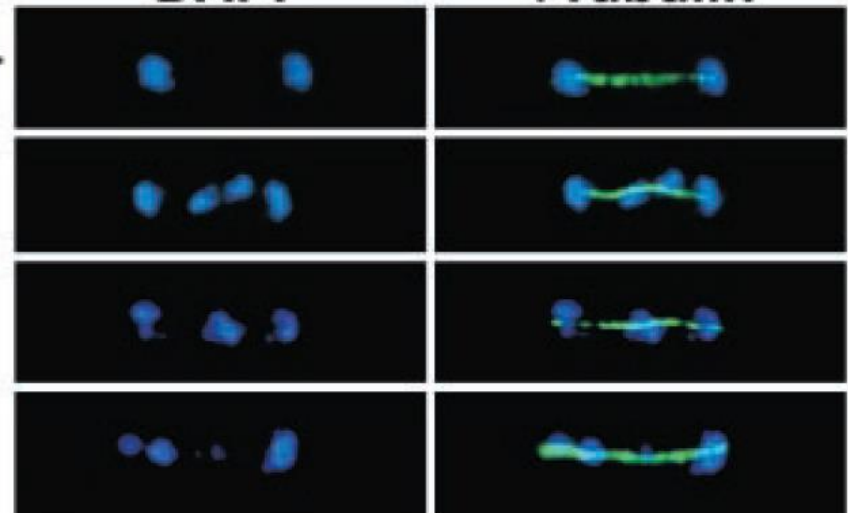
+Tubulin

WT

*ago1*Δ

*dcr1*Δ

*rdp1*Δ



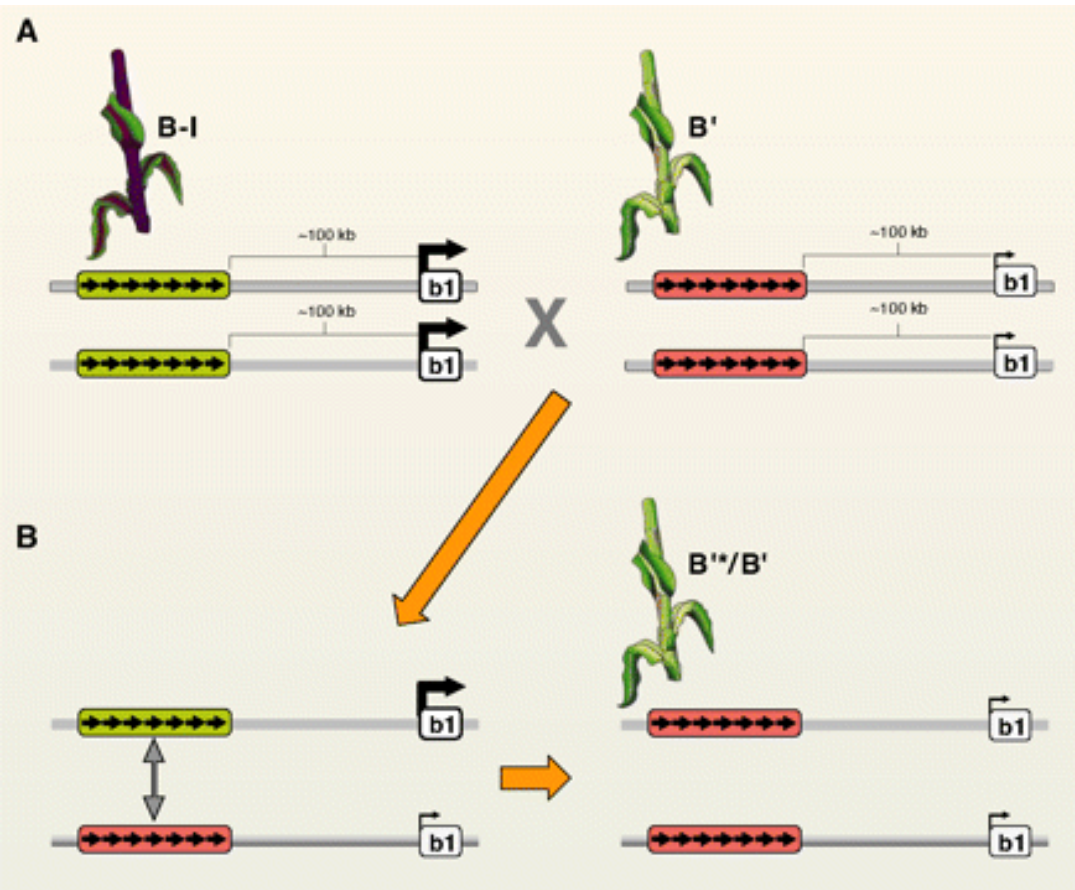
Dcr, Ago mutants show
Accumulation of long non coding centromeric
RNAs

Dcr, Ago, and RdRP mutants show loss of
centromeric siRNAs and
centromere instability
and missegregation of chromosomes

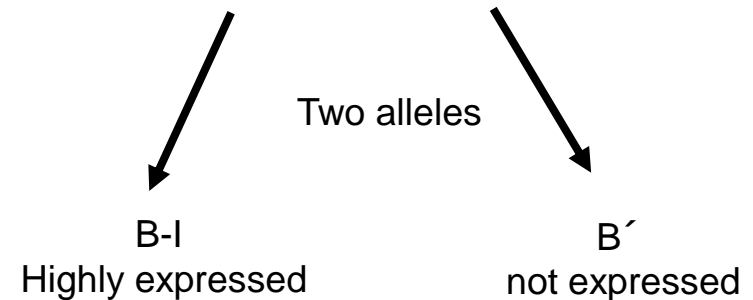
Examples RNA mediated epigenetic crosstalk

Paramutation as an example for Co-transcriptional silencing of protein coding genes.

Paramutation in maize



B1-gene encodes for a protein of the Anthocyanidin pathway responsible for purple pigments



Heterozygous F1 show silencing of the B-I allele caused by small RNAs derived from the silent B' allele

... is an interaction between two alleles at a single locus, whereby one allele induces a heritable change in the other allele