



Course Title:

Epigenetics

Lecture Titles:

Lecture I: General Overview and History of Epigenetics

Lecture II: DNA methylation

Lecture III: Alteration in DNA methylation and its transgenerational inheritance

Lecture IV: DNA methylation and genome stability

Lecture V: Epigenetic variation in genome evolution and crop improvement

Lecture VI: Histone modifications

Lecture VII: Small RNA and RNA interference

Lecture VIII: Epigenetics and gene expression

Lecture : TBD

Lecture : TBD

Lecture : Summary

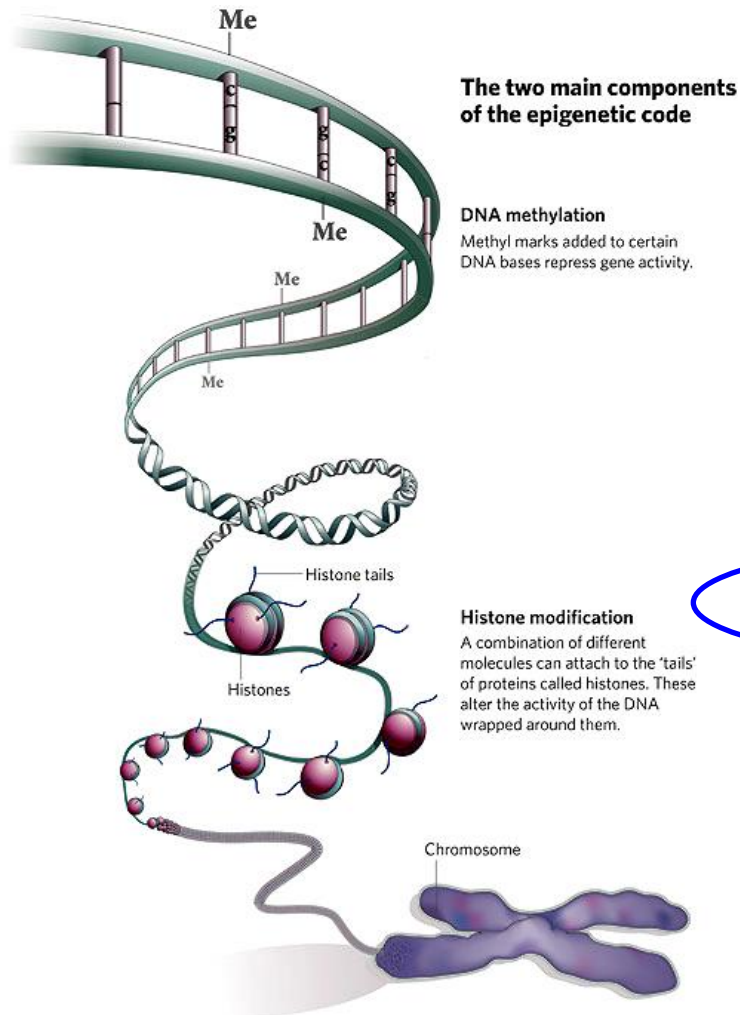
Lecture VII:

Small RNA and RNA interference

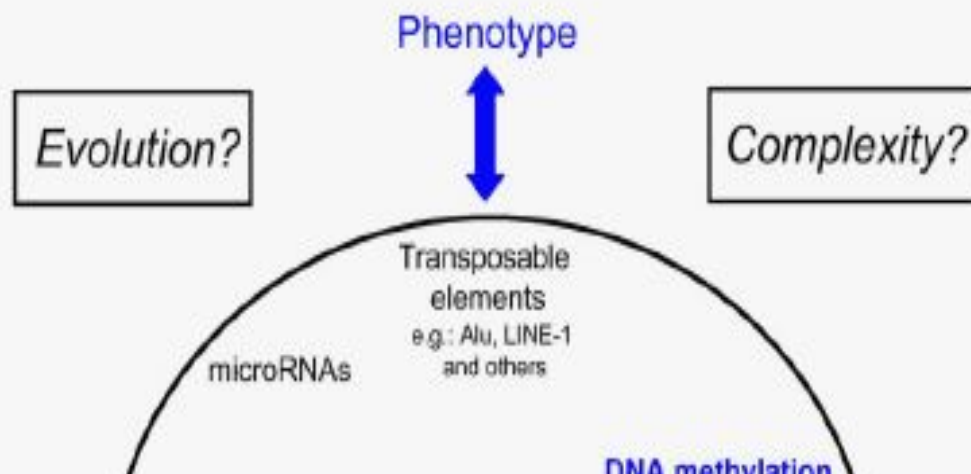
Ningning Wang

1/11/2012

Molecular biology research



- Sequences variation and inheritance
- DNA sequences(C, A, T, G)
- Chromatin modifications variation
 - DNA methylation
 - histone modifications
 - small RNA variation



Review

Non-coding RNAs, epigenetics and complexity

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Fig. 2. Epigenetics as a central molecular mechanism. Epigenetic mechanisms work in concert with genetic mechanisms and they can have different phenotypes that

(molecule), the environment and all factors acting on environmental changes, leading to a new concept.

How did they work with small RNAs?

RNAi !

A 3D molecular model illustrating the RNA interference mechanism. The background shows a complex, multi-colored protein structure in shades of blue, green, and brown. In the foreground, several double-stranded RNA molecules are depicted as colorful chains of beads (red, yellow, green, blue, purple) forming a double helix. One of these RNA molecules is shown interacting with a specific region of the protein structure.

RNA Interference: Ancient Mechanism With A Promising Future

Gene Silencing!

RNAi Timeline

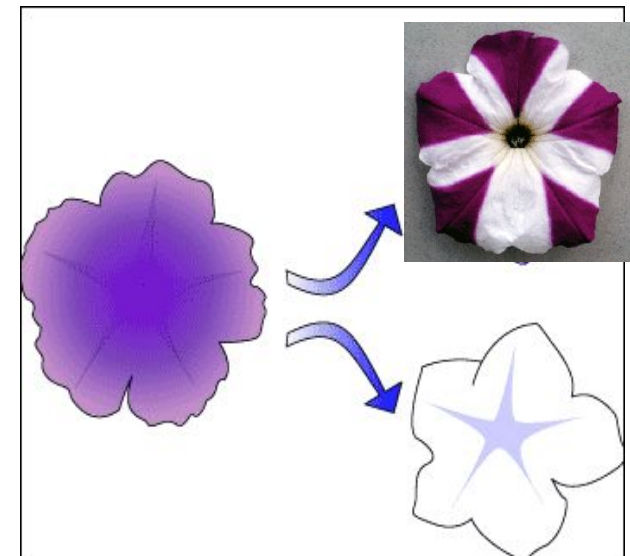
- **1990** co-suppression of purple color in plants.
- **1995** Guo S, and Kemphues KJ. First noticed that sense RNA was as effective as antisense RNA for suppressing gene expression in worm *C. elegans*
- **1998** Fire et al. First described RNAi phenomenon in *C. elegans* by injecting dsRNA into *C. elegans* which led to an efficient sequence-specific silencing and **coined the term "RNA Interference"**.
- **2000** Zamone et al. Reported processing of long dsRNA by Rnase III (**Dicer**) into shorter fragments of 21-23-nt intervals in *Drosophila* extracts
- **2001** Bernstein et al. Cloned Dicer, the RNase III enzyme that is evolutionarily conserved and contains helicase and PAZ domains, as well as two dsRNA-binding domains.
- **2002** Tuschl T and colleagues First described RNAi in mammalian cells
- **2003** Paddison et al. Sui et al. Paul et al. Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells.
- **2003** Song et al. First reported that siRNAs can be used therapeutically in whole animals
- **2004** Kawasaki and Taira Morris et al. First observed that siRNA silences gene at transcriptional level possibly through directing *de novo* DNA methylation.
- **2006** Nobel Prize in Medicine to Andrew Z. Fire (Stanford University) and to Craig C. Mello (University of Massachusetts Medical School) for their discovery of RNA interference - gene silencing by double-stranded RNA
- **2007** Smith, L.M., Pontes, O., Searle, I., Yelina, N.E., Yousafzai, F.K., Herr, A.J., Pikaard, C. and Baulcombe, D. A novel SNF2 protein associated with nuclear RNA silencing and spread of a silencing signal between cells in *Arabidopsis*.

Co-suppression: Exogenous gene suppress homologous endogenous genes

- **1990 Richard Jorgensen and his group discovered gene silencing while working with petunias.**

Petunias developed areas of hypopigmentation when transduced with the gene encoding a protein required for

Gene made
gene silencing



<http://www.pbs.org/wgbh/nova/sciencenow/3210/02.html>

<http://ag.arizona.edu/pls/faculty/jorgensen.html>

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

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dsRNA made gene silencing

- ◆ Silencing of a green fluorescent protein (GFP) reporter in *C. elegans* occurs when animals feed on bacteria expressing GFP dsRNA (a) but not in animals that are defective for RNAi (b).



The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"



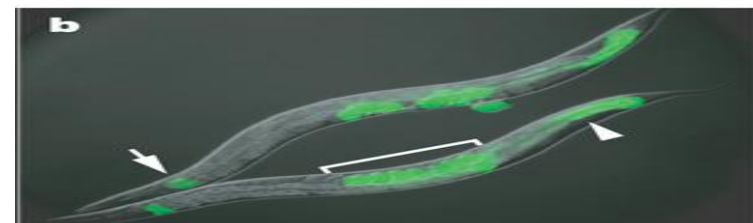
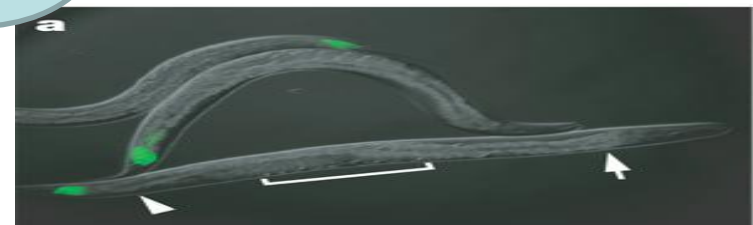
Photo: Stanford

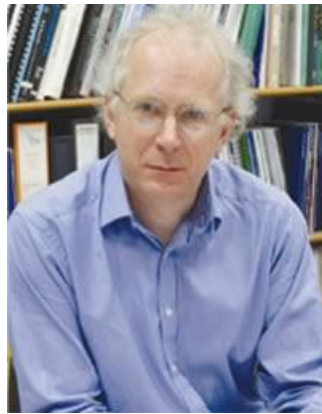
Andrew Fire



Photo: UMASS

Craig C. Mello



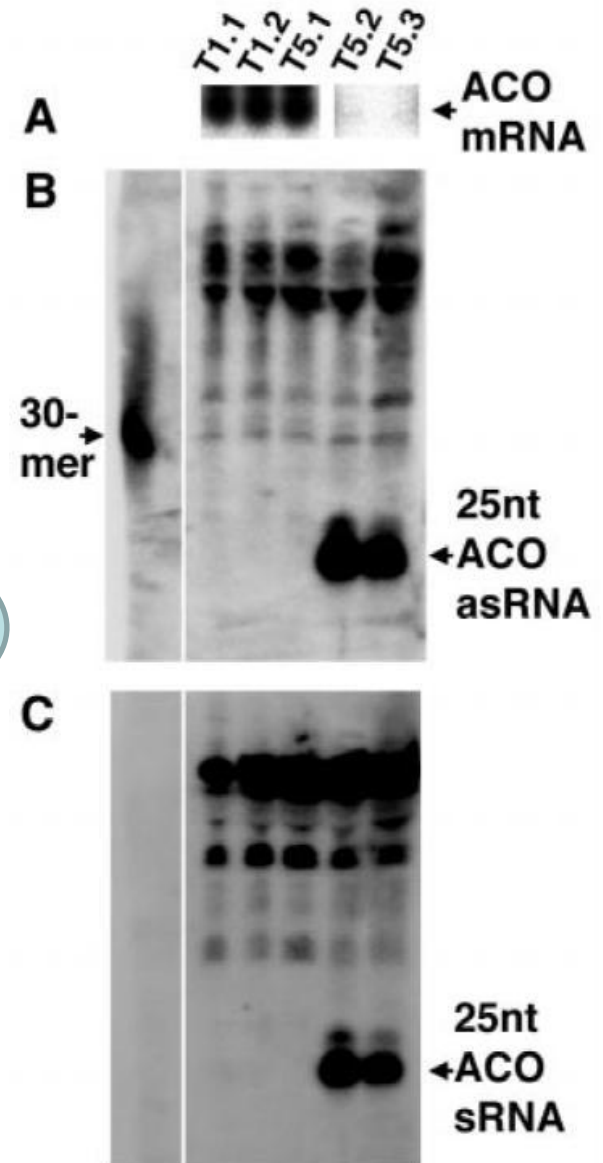


A Species of Sense or anti-sense sRNA in Gene Silencing

Andrew Fire

Sense or anti-sense sRNA made gene silencing

Posttranscriptional gene silencing (PTGS) is a sequence-specific defense mechanism that can target both cellular and viral mRNAs. Here, three types of transgene-induced PTGS and one example of virus-induced PTGS were analyzed in plants. In each case, antisense RNA complementary to the targeted mRNA was detected. These RNA molecules were of a uniform length, estimated at 25 nucleotides, and their accumulation required either transgene sense transcription or RNA virus replication. Thus, the 25-nucleotide antisense RNA is likely synthesized from an RNA template and may represent the specificity determinant of PTGS.



Small RNAs as big players in plant abiotic stress responses and nutrient deprivation

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Abiotic stress is one of the primary causes of crop losses worldwide. Much progress has been made in unraveling the complex stress response mechanisms, particularly in the identification of stress responsive protein-coding genes. In addition to protein coding genes, recently discovered microRNAs (miRNAs) and endogenous small interfering RNAs (siRNAs) have emerged as important players in plant stress responses. Initial clues suggesting

proteins (*dcl1*, *hen1*, *hyl*, *se* and *hst*) required for miRNA generation and miRNA target genes (*phb* and *ago1*) were first identified through genetic screens for developmental defects [9–15]. A comprehensive examination of miRNAs in plant development is provided by several excellent recent reviews [5–8,16–18]. In this review, the emerging roles of miRNAs and endogenous siRNAs in plant stress responses are discussed.

After the Hamilton and Baulcombe report in 1999, short RNAs were soon found in all other systems where dsRNA was able to induce gene silencing.

Small interfering RNAs were soon found to not only induce mRNA degradation, but also guide other processes, including inhibition of translation, cytosine methylation, DNA elimination, and heterochromatin formation.

Most processes known to be controlled by RNAi include synthesis of dsRNA from DNA or RNA, cleavage of dsRNAs by distinct RNases into small RNAs, small RNAs then are involved in specific biological processes.

The greatest impact of RNAi is its application as an experimental tool to knock down gene expression in cells and intact organisms.

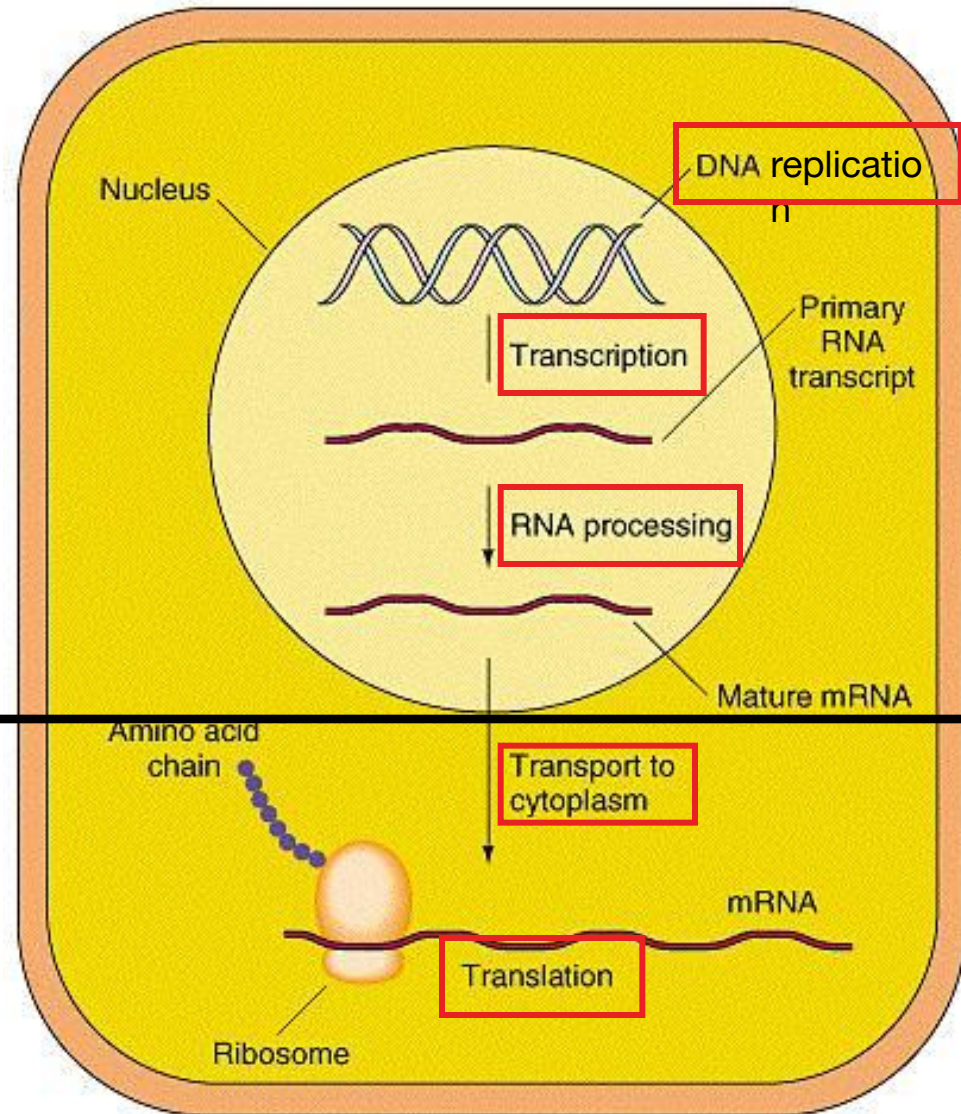
How did small RNA work?

PTGS!

Compartmentalization of processes that necessitates transport

transcriptional gene silencing (TGS)

post-transcriptional gene silencing (PTGS)



Gene Silencing

- Term used to describe **switching off of a gene** that would normally be turned on, i.e. silencing a gene that would normally be expressed.
- Can be transcriptional (TGS) (DNA methylation, histone modification) or post-transcriptional (PTGS) (RNAi) .

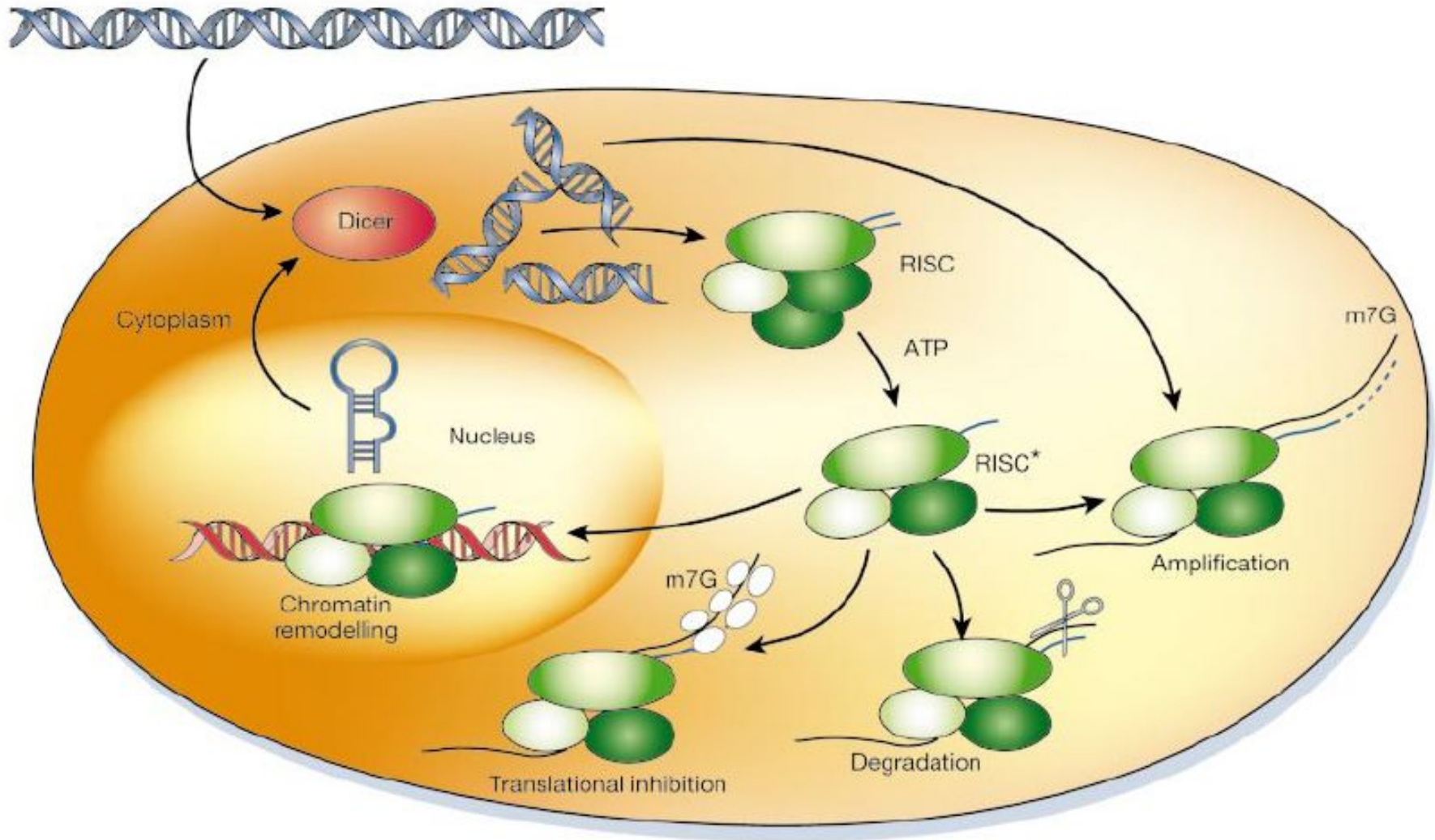
Post-transcriptional gene silencing (PTGS)

- It identifies long double stranded RNA molecules (dsRNA) such as those generated during virus replication, transposons mobilization or aberrant RNA synthesis.
- These molecules act as trigger signals for the PTGS machinery, guiding the cell to an alert state-and the dsRNA molecules are destroyed

RNAi (PTGS)

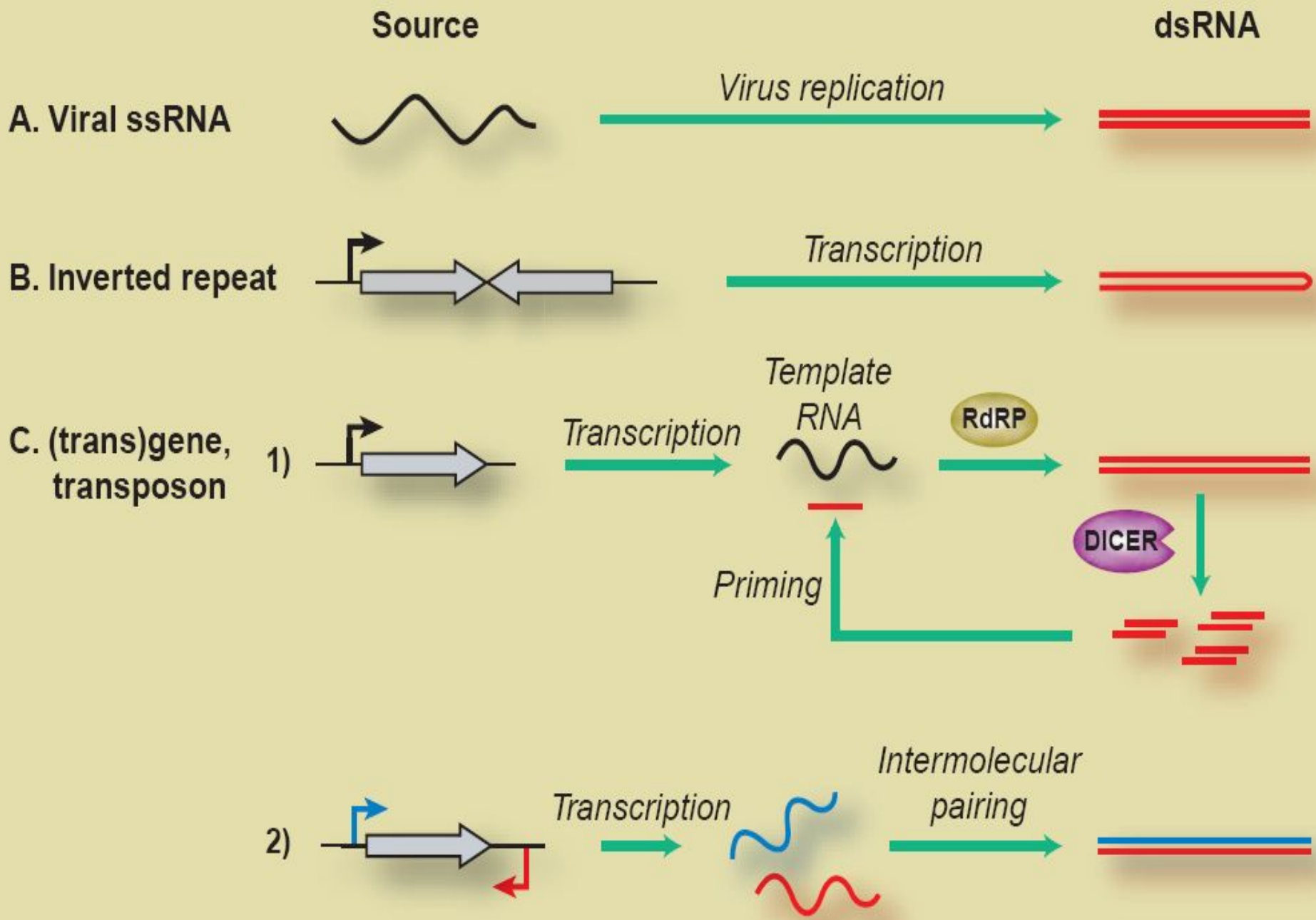
- **Gene silencing mechanisms have since been found in many organisms including plants, fungi, worms, Drosophila, mice and humans**
- **The best characterized mechanism of post transcriptional gene silencing is RNA interference or RNAi**

The effects of a dsRNA in a cell



How did small RNA
generate?

Exogenous or endogenous!

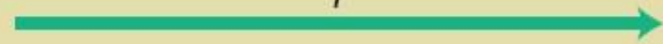


Source

dsRNA

A. Viral ssRNA

Virus replication



B. Inverted repeat

Transcription



C. (trans)gene, transposon

1)

Template RNA

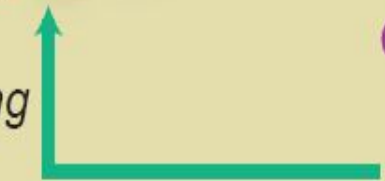
Transcription

RdRP



Priming

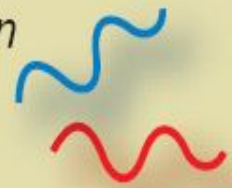
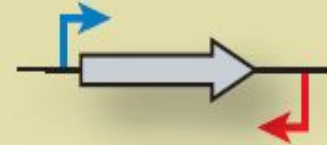
DICER



2)

Intermolecular pairing

Transcription



Now.....

- Small RNA: non_coding, ssRNA, 18-30nt
 - rasiRNA
 - miRNA
 - nat-siRNA
 - ta-siRNA
 - lsiRNA (>30nt)

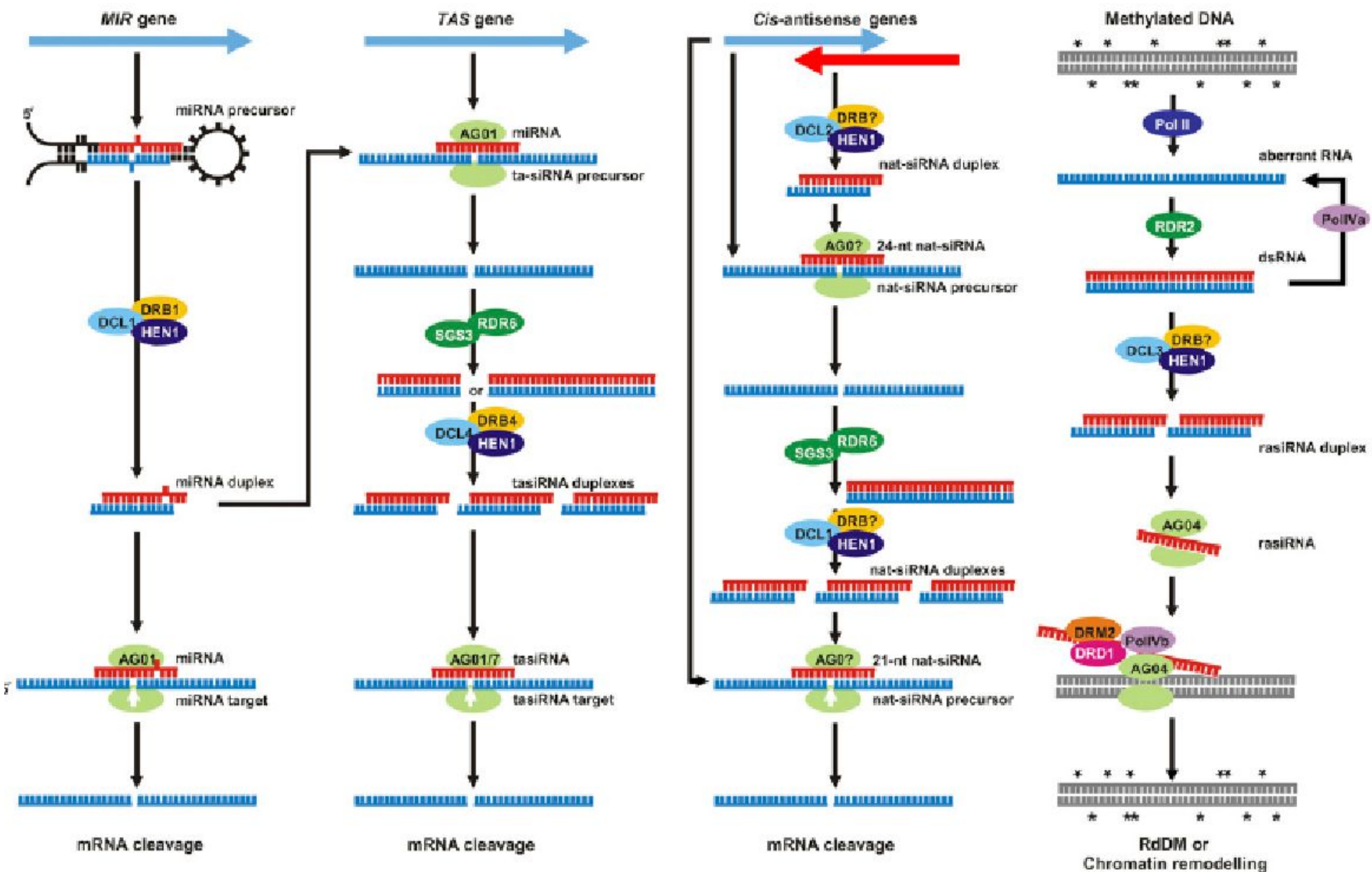


Figure 3. The parallel RNA silencing pathways of Arabidopsis. Schematic representation of the parallel DCL/sRNA-directed RNA silencing pathways in the model dicotyledonous species Arabidopsis, outlining the specific step or steps in each pathway for the individual RNA silencing-associated proteins mentioned in the text of this review.

DICING AND SLICING

- The basic pathway of RNA silencing is similar in many animals and plants. An **RNA dependent RNA polymerase (RdRP)** generates double stranded RNA from a single stranded precursor. The double stranded RNA is cleaved into 21-25nt fragments by nucleases of the **Dicer** family. These RNA fragments, known as micro (mi)RNAs and short interfering (si)RNAs, are separated into single stranded molecules and one of the two strands then becomes bound to an **Argonaute nuclease**. This RNA then forms base paired duplex structures with longer RNAs and thereby guides Argonaute to its target. Argonautes are sometimes referred to as **Slicer**. RNA silencing is therefore a combination of Dicing and Slicing.

Key RNAi proteins in different model organisms

| | Dicer | Argonaute | RdRP |
|-----------------------|-------|-----------|------|
| <i>S.cerevisiae</i> | 0 | 0 | 0 |
| <i>S.pombe</i> | 1 | 1 | 1 |
| <i>C.elegans</i> | 1 | 27 | 4 |
| <i>D.melanogaster</i> | 2 | 5 | 0 |
| <i>A.thaliana</i> | 4 | 10 | 6 |
| <i>H.sapiens</i> | 1 | 8 | 0 |

Transcriptional silencing and epigenetics

The siRNA targeting mechanism is best understood in systems in which RNA is the target and the **silencing is posttranscriptional**. However there are also systems in which the targeting mechanism targets DNA and the silencing mechanism operates at the **transcriptional level** (Jones *et al.*, 2001).

In these systems the **transcriptional silencing is associated with methylation of the target DNA or modification of its associated histones or both**.

The transcriptional silencing effect in these systems may persist for longer than the initiator silencing RNA and, in extreme examples, for many generations (Jones *et al.*, 2001).

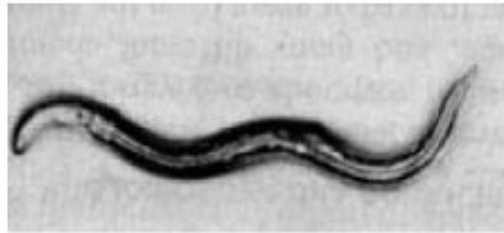
These are examples therefore in which RNA triggers a **transgenerational effect**. Because this is a heritable effect without changes to the sequence of DNA it is **epigenetic rather than genetic**.

microRNAs

MicroRNAs (miRNAs)



A. thaliana/
O. sativa
>80 miRNAs



C. elegans
>100 miRNAs



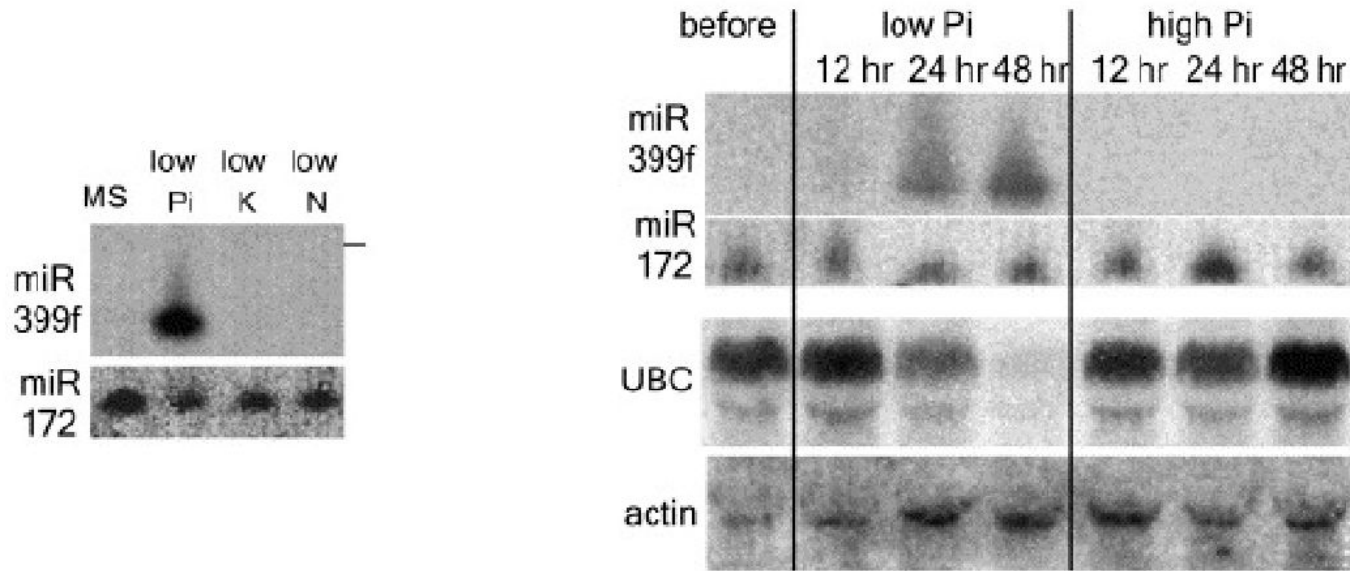
D. melanogaster
>100 miRNAs



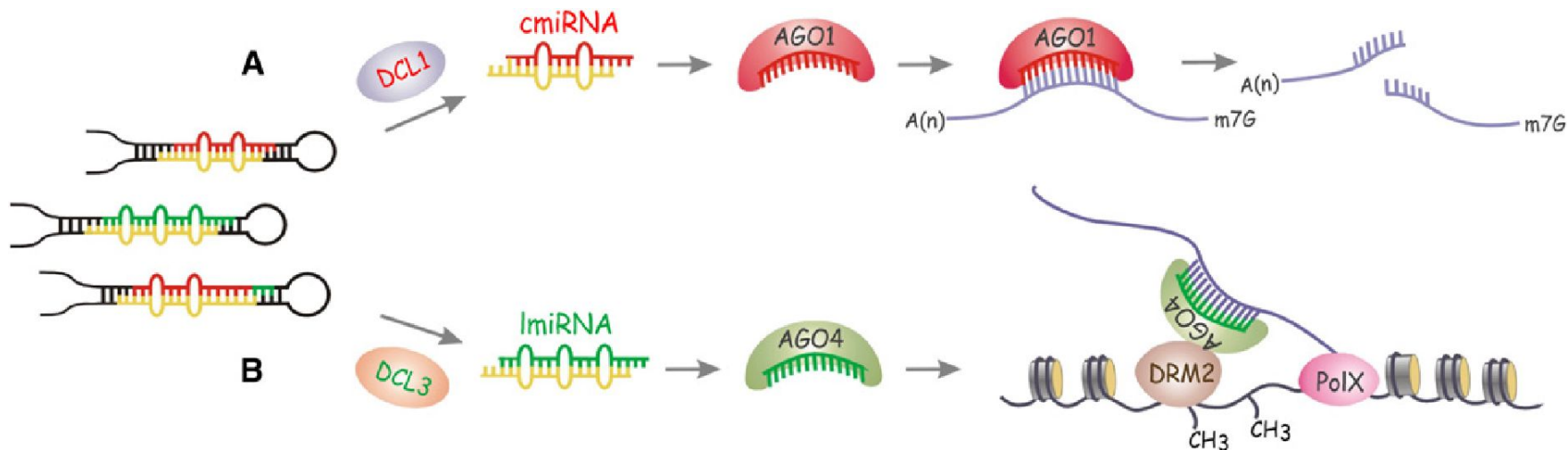
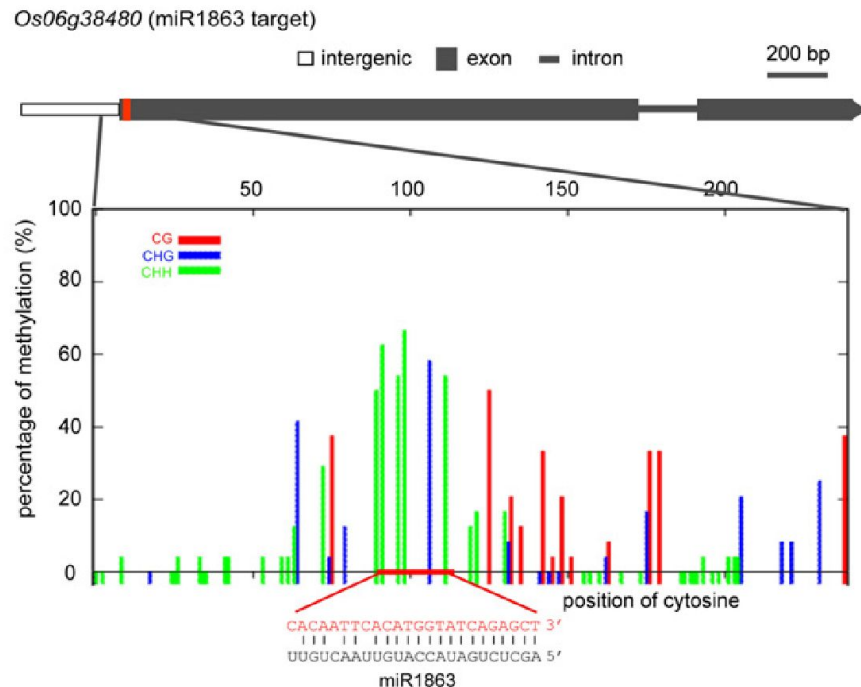
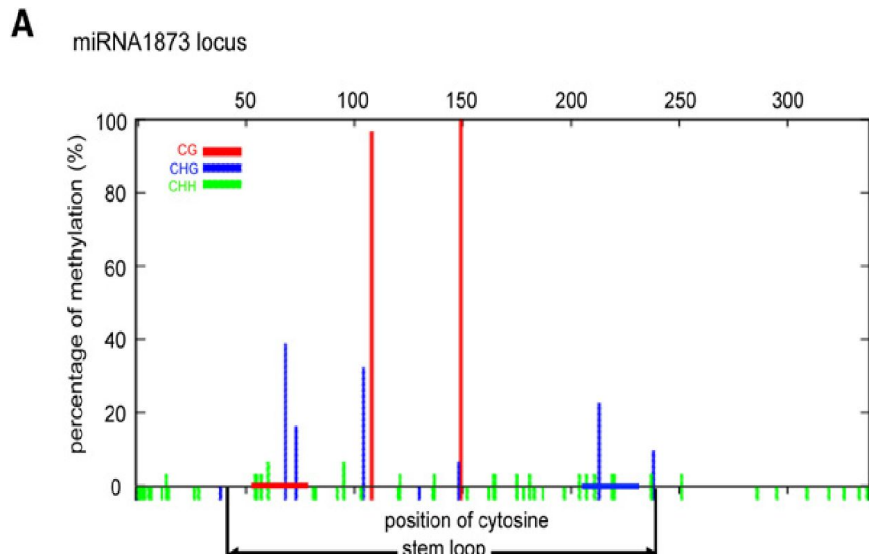
H. sapiens/
M. musculus
>200 miRNAs

See the RNA Family database—<http://www.sanger.ac.uk/software/rfam/>

miRNA-controlled stress adaptation



Chiou et al., 2006
Fujii et al., 2005



heterochromatic siRNAs
hc-siRNAs

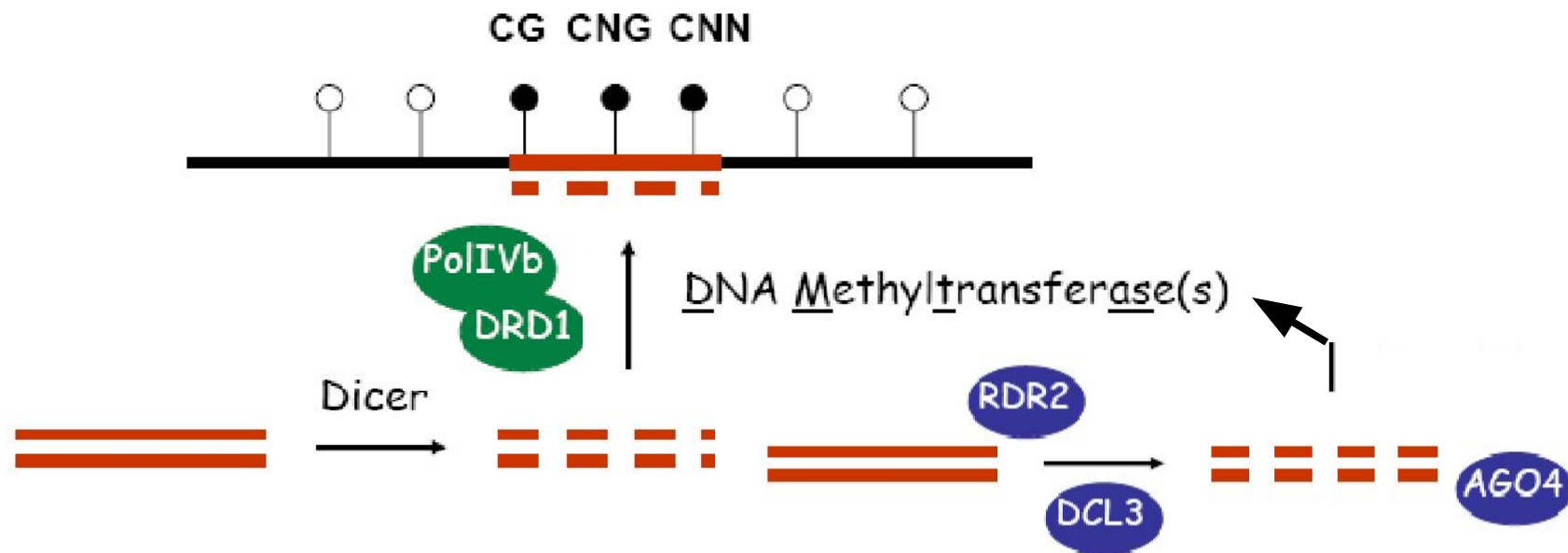
rasRNA

Arabidopsis, repeat sequences have been shown to be an extremely rich source of a unique class of siRNAs, termed rasRNAs, which are of the 24-nucleotide size class, and rasRNAs have been suggested to direct DNA methylation and hence to transcriptionally silence repetitive DNA sequences in the plant genome (Chan et al., 2005). Wassenecker and colleagues (1994)

raSiRNA

- Heterochromatin found near centromeres and telomeres are largely composed of **short tandem DNA repeats and transposable** elements. Heterochromatin structure is plastic and had the ability to regulate the expression of near by genes.
- **raSiRNA Can**
 1. **Shut down expression of transposable elements.**
 2. **Histone methylation.**
 3. **DNA methylation modification.**
- raSiRNA functions via partially overlapping the components of siRNA pathway.
- Instead of loading into **RISC**, raSiRNA load another complex called **RITS (Inducible Transcriptional Silencing)**. Produced by the dicerlike **DCL3** in *A. thaliana*.

Characteristics of RNA-directed DNA methylation (RdDM)



- ✎ RNA-DNA sequence identity: base pairing
- ✎ 30 bp DNA target (nucleosome: 147 bp DNA)
- ✎ Not just CG
- ✎ If dsRNAs contain promoter sequences → TGS/^mC-pro

dsRNA can also interact with DNA and induce epigenetic changes...

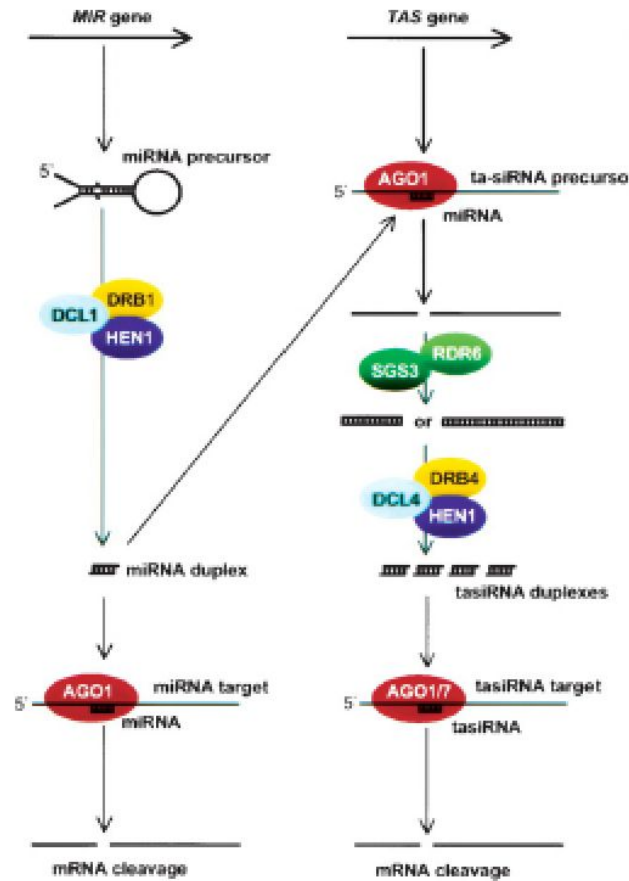
Epimutations:

- heritable, but potentially reversible changes in gene expression **without** alterations in DNA sequence (i.e. classical mutations)
- caused by changed chromatin state (DNA methylation, histone modification, nucleosomal positioning, etc.)

RNAi-mediated heterochromatin formation
RNA-directed DNA methylation

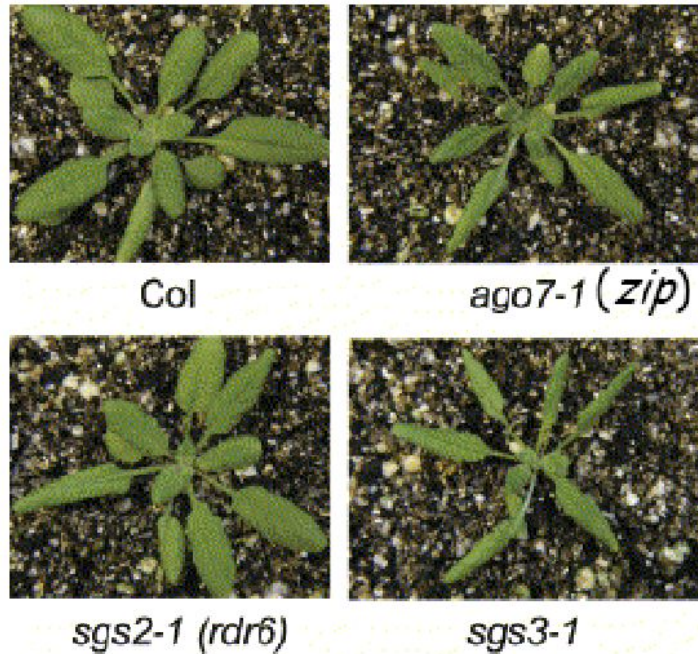
trans-acting siRNAs
(ta-siRNAs)

trans-acting siRNAs (ta-siRNAs)



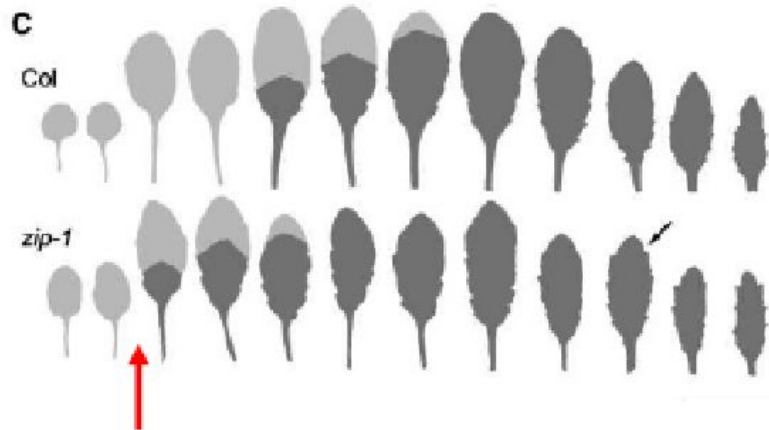
Vaucheret 2006, *Genes Dev.* 20, 759-771.

ta-siRNAs are involved in leaf development and polarity

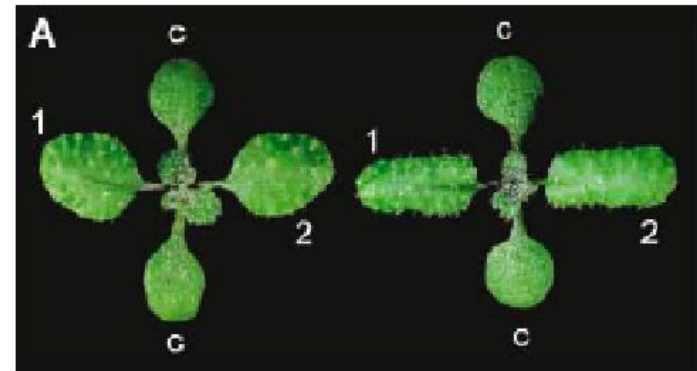


Vazquez et al. 2004, Mol. Cell 16, 69-79

Mutation of an Argonaute gene enhances phase change



ZIP = AGO 7



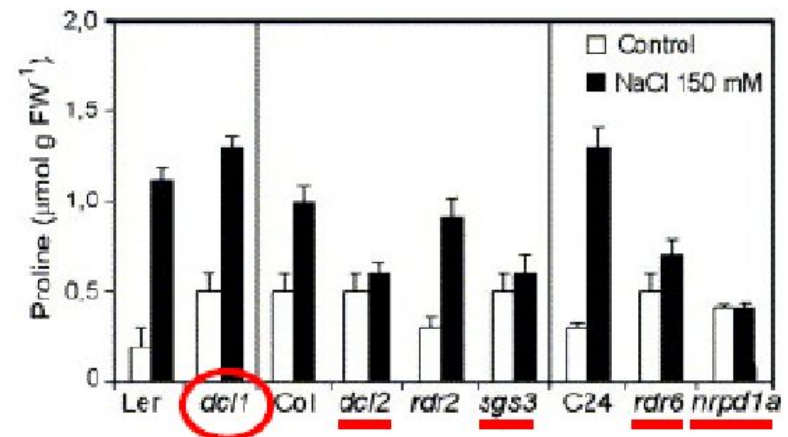
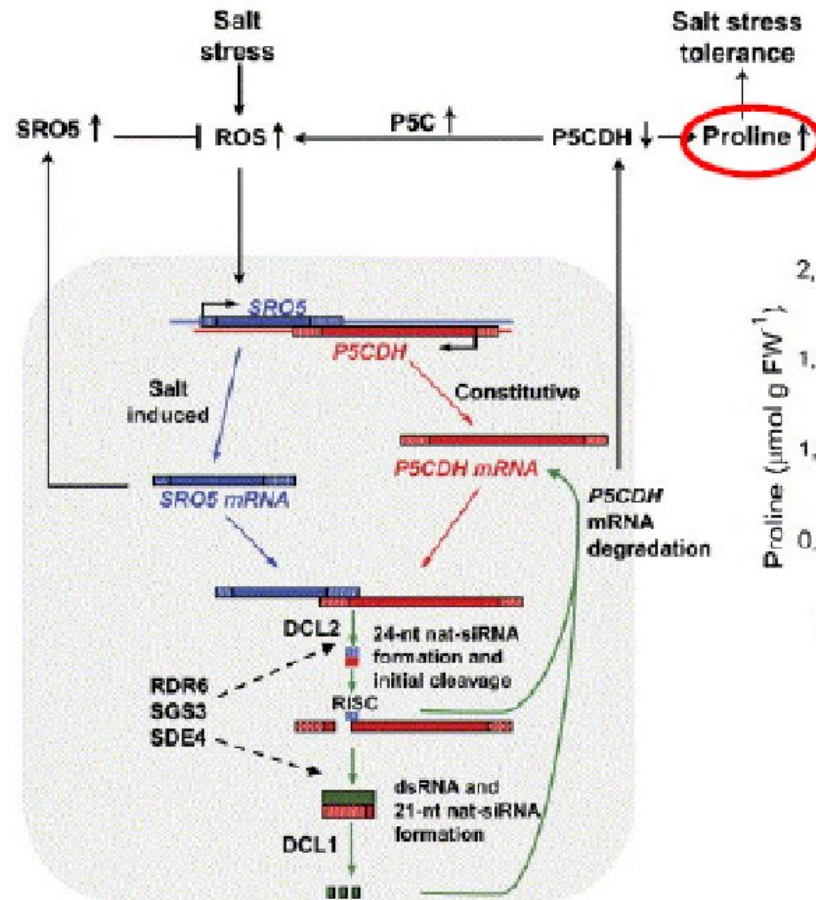
WT

zip-1

Hunter et al. 2003

natural antisense transcript siRNAs
(nat-siRNAs)

nat-siRNAs act in the plant response to salt stress



Borsani et al. 2005, Cell 123, 1279-1291

Evidence for nat-siRNAs formation from other biotic and/or abiotic stress inducible convergent overlapping gene pairs in *Arabidopsis*

RNA interference

- RNA-RNA interaction in the **cytoplasm**
- RNAi regulates gene expression at the posttranscriptional level
- Regulation of mRNAs occurs via degradation and/or translational inhibition
- RNAi is conserved across kingdoms

Organisms capable of RNAi:

Fungi e.g. *Neurospora crassa*
fission yeast
but: lost in *S. cerevisiae*

Plants

Invertebrates (e.g. *Drosophila*
Caenorhabditis)

Mammals

thought



Plants have an elaborate small RNA-based silencing system, connected to aspects of plant life-style? (sessile, lack of adaptive immune system, postembryonic development, etc.)



Many plant small RNAs are made by the redundant and/or cooperative action of different Dicers and are under feed-back control; robust as well as fine-tuneable system



Plant small RNAs can trigger silencing on the post-transcriptional as well as transcriptional level



Post-transcriptional RNA silencing in plants is not cell-autonomous; RdRPs!



Plant viruses have evolved counterstrategies to evade plant RNA-based immune system

can hope that RNA silencing-based technologies will help humankind to face the challenges of productive agriculture in the increasingly unfavorable environmental conditions associated with climate change.

Thank you for your attention