NORTHEAST NORMAL UNIVERSITY 1946 东北师范大学

Course Title:

Epigenetics

Lecture Titles:

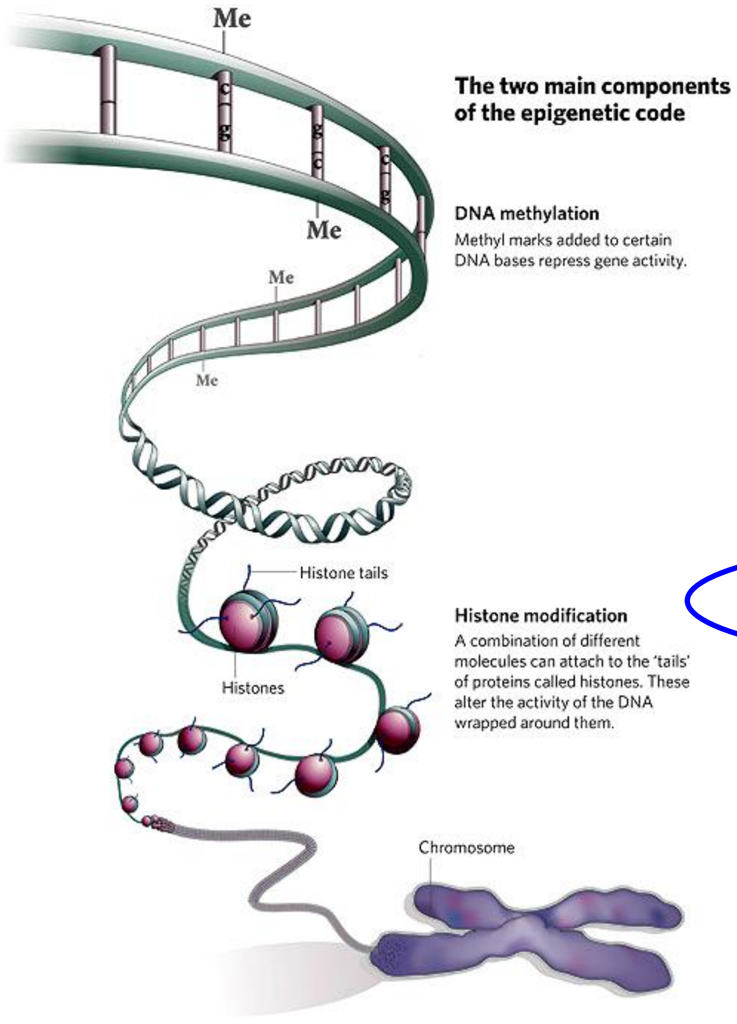
Lecture I: General Overview and History of Epigenetics
Lecture II: DNA methylation
Lecture III: Alteration in DNA methylation and its transgenerational inheritance
Lecture IV: DNA methylation and genome stability
Lecture V: Epigenetic variation in genome evolution and crop improvement
Lecture VI: Histone 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



The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

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

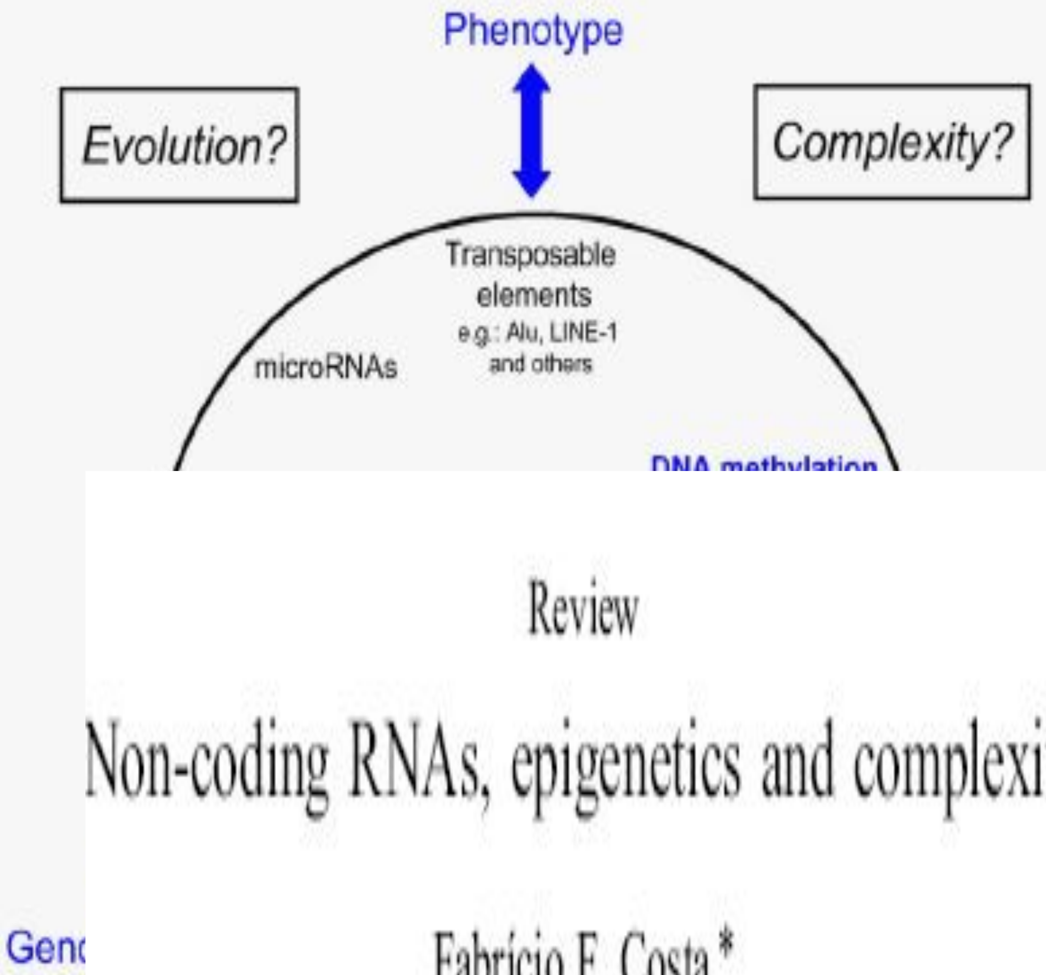


Fig. 2. Epigenetics as a central molecular ecule), the environment and all
epigenetic mechanisms work in concert ing on environmental changes,
they can have different phenotypes that concept.

Review

Non-coding RNAs, epigenetics and complexity

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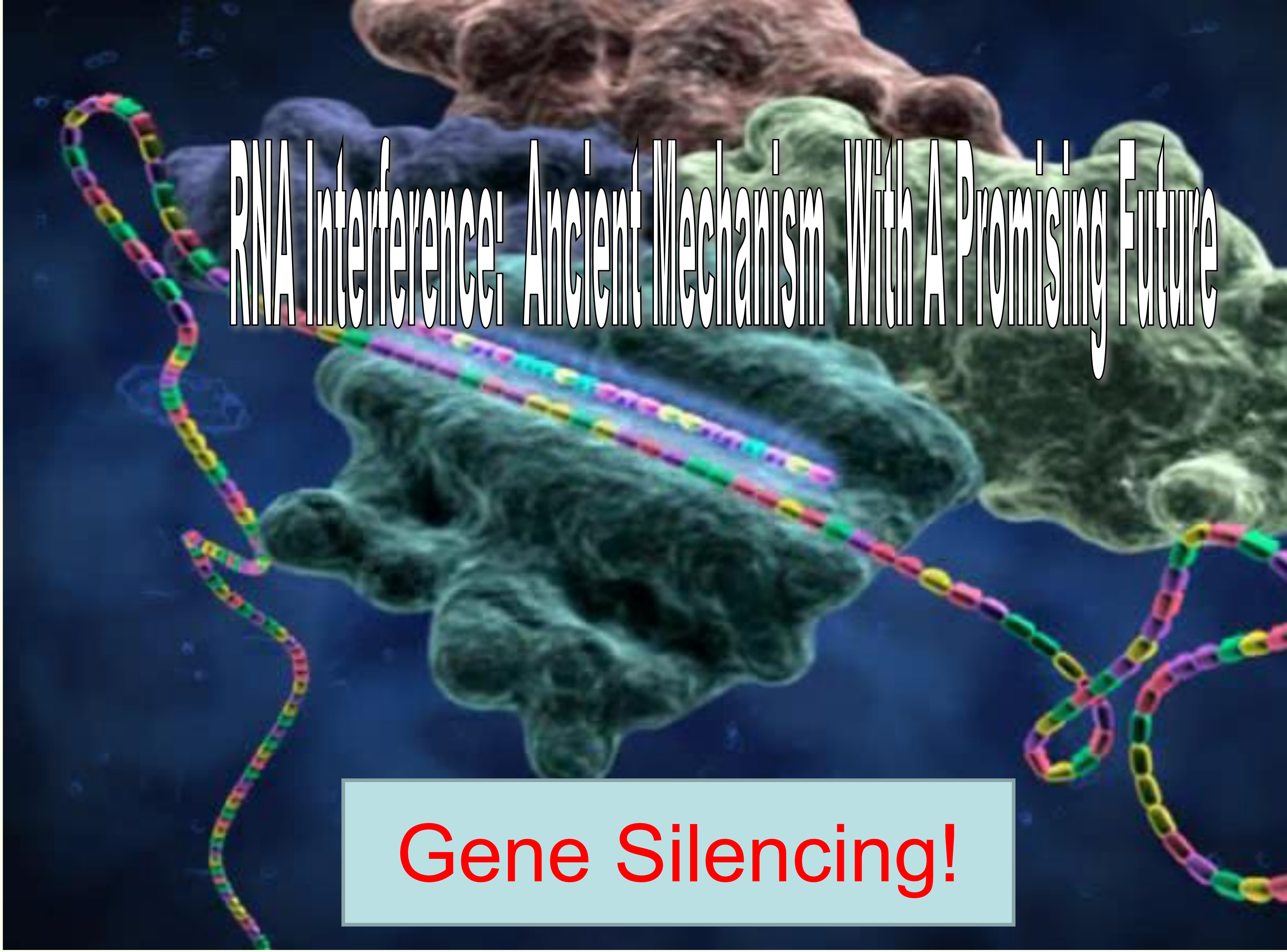
Received 18 August 2007; received in revised form 26 November 2007; accepted 7 December 2007

How did they work with small RNAs?

RNAi !

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*.

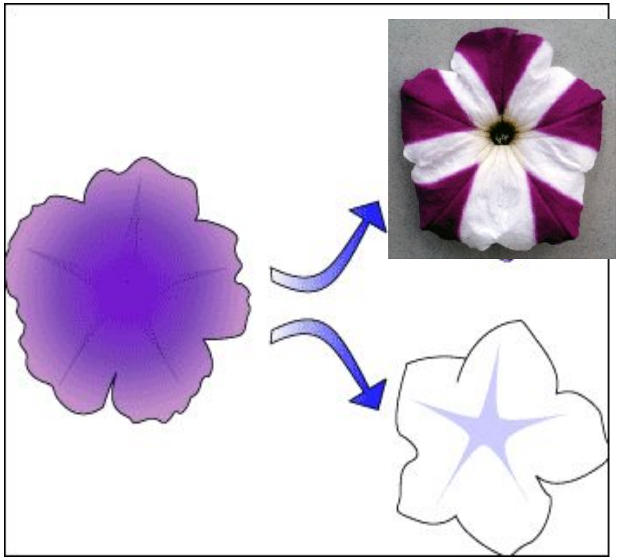
Co-suppression: Exogenous gene suppress homologous endogenous genes

- **1990 Richard Jorgensen and his group discovered gene silencing while working with petunias.** Petunias developed areas of hypopigmentation when transduced with the gene enc
  required f

  ungi.

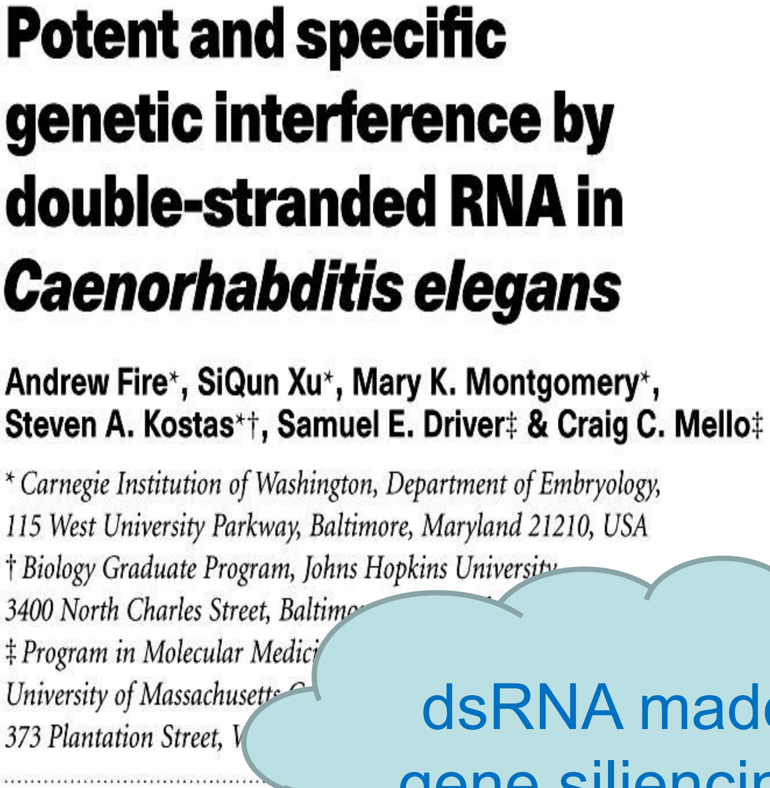
  called

Gene made
gene siliencing



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*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*,
Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

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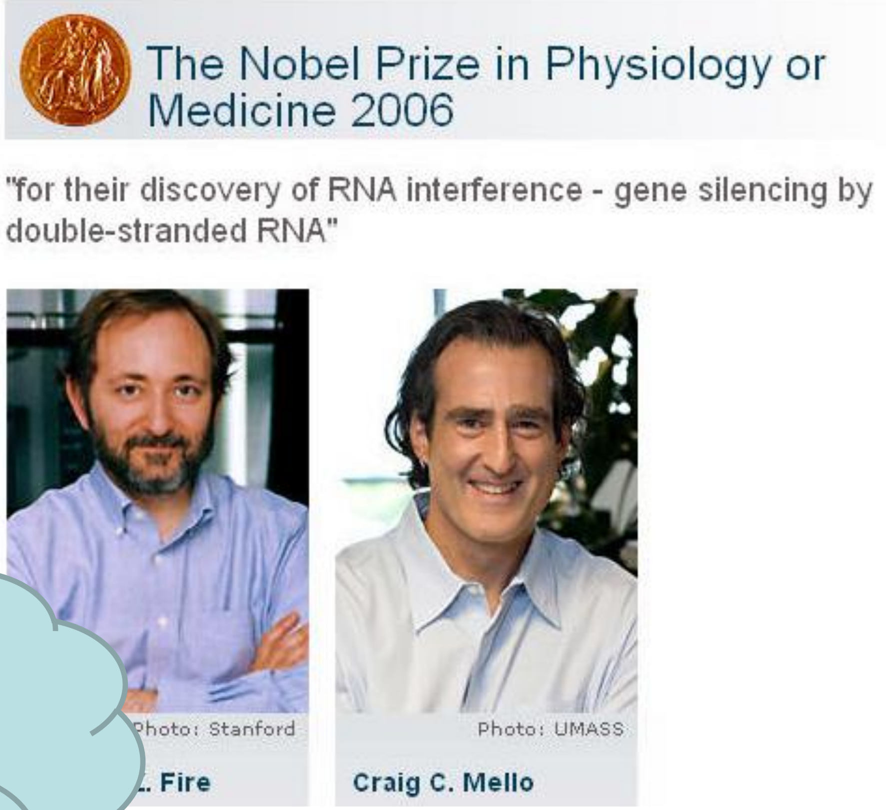
115 West University Parkway, Baltimore, Maryland 21210, USA
† Biology Graduate Program, Johns Hopkins University
3400 North Charles Street, Baltim
‡ Program in Molecular Medic
University of Massachusett
373 Plantation Street, V

dsRNA made
gene siliencing

- Silencing of a green fluo 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

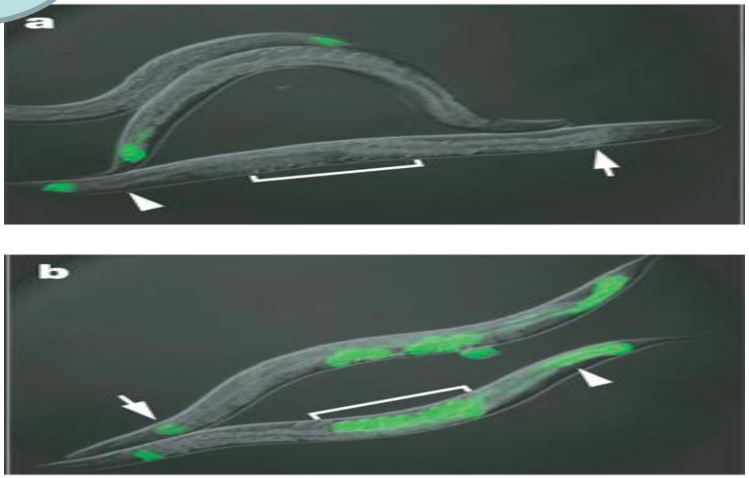
"for their discovery of RNA interference - gene silencing by double-stranded RNA"

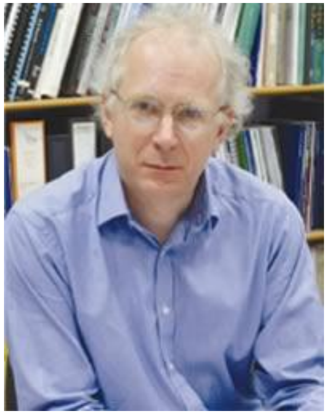
Photo: Stanford

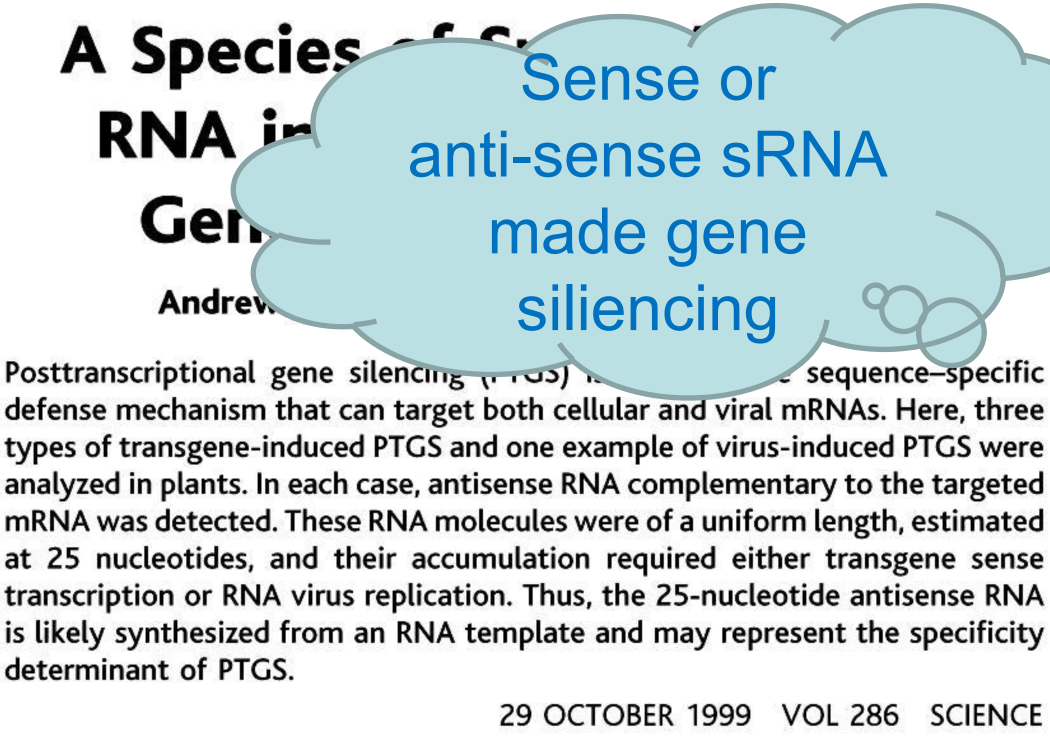
. Fire

Photo: UMASS

Craig C. Mello







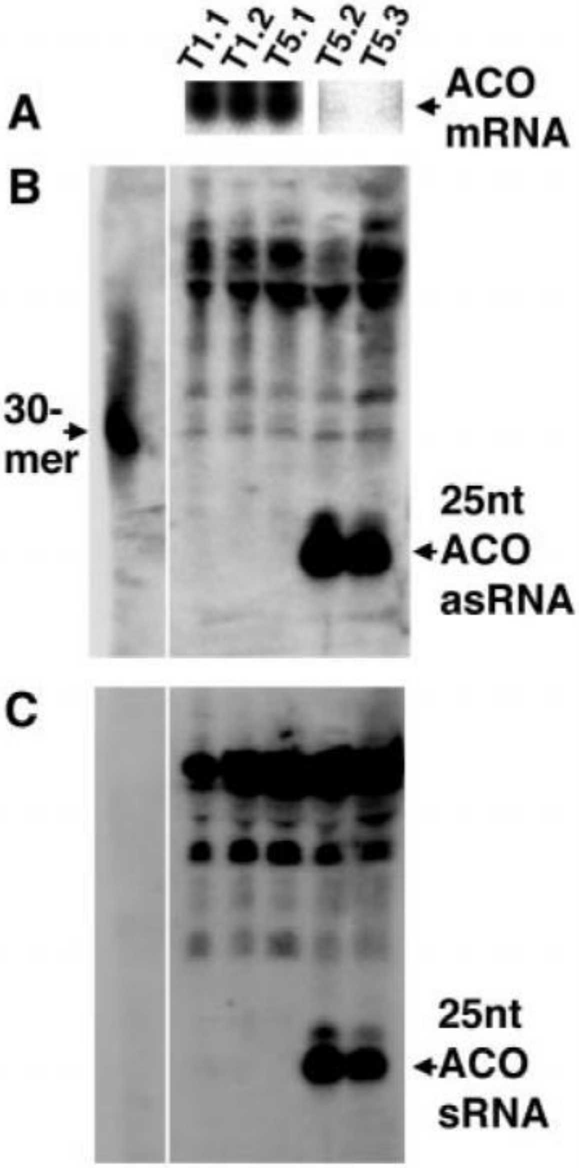
A Species
RNA i
Ge

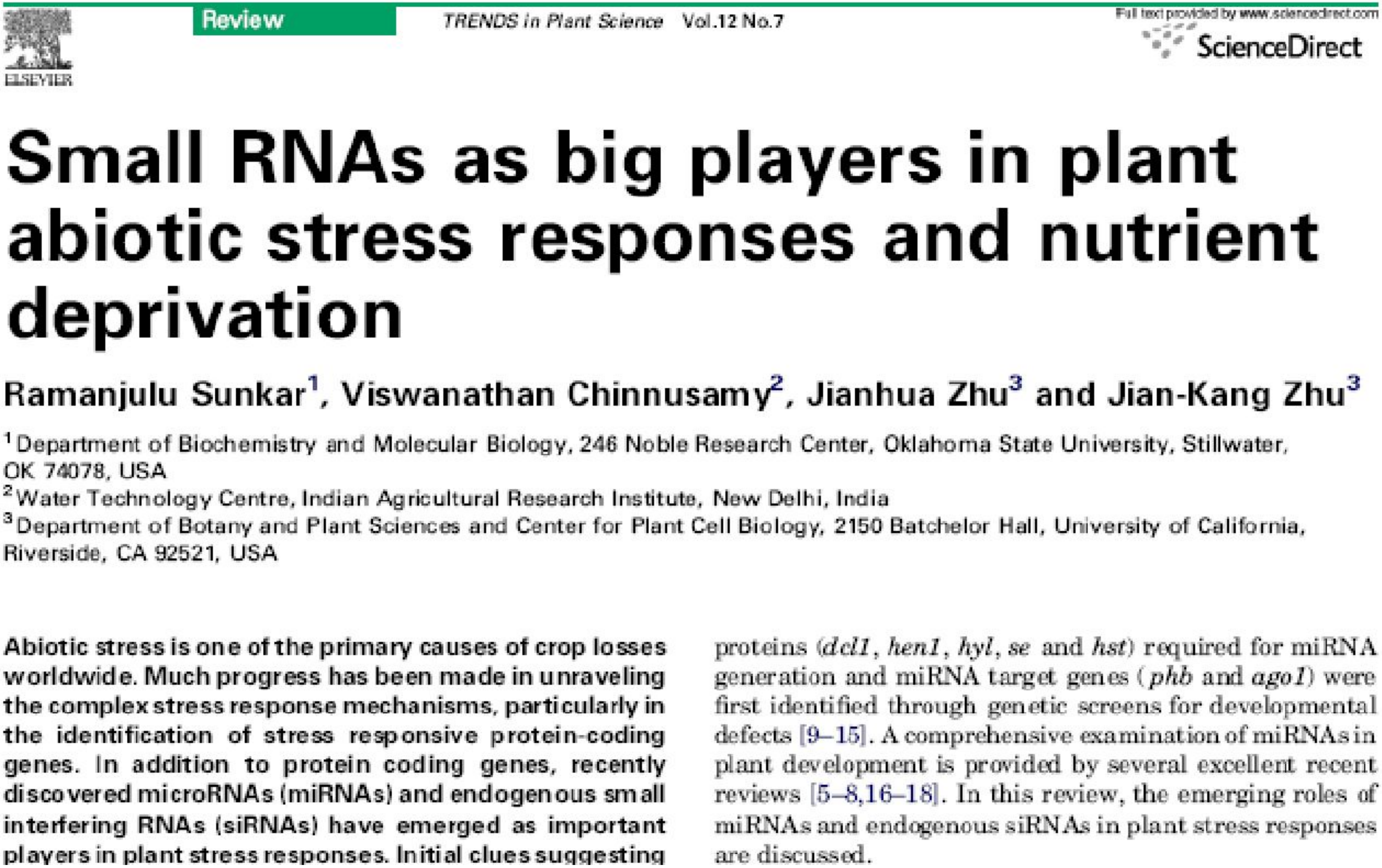
Andrew

Posttranscriptional gene silencing (PTGS) i 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.

29 OCTOBER 1999 VOL 286 SCIENCE

Sense or anti-sense sRNA made gene siliencing





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

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[3] Department of Botany and Plant Sciences and Center for Plant Cell Biology, 2150 Batchelor Hall, University of California, Riverside, CA 92521, USA

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.

2008

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.

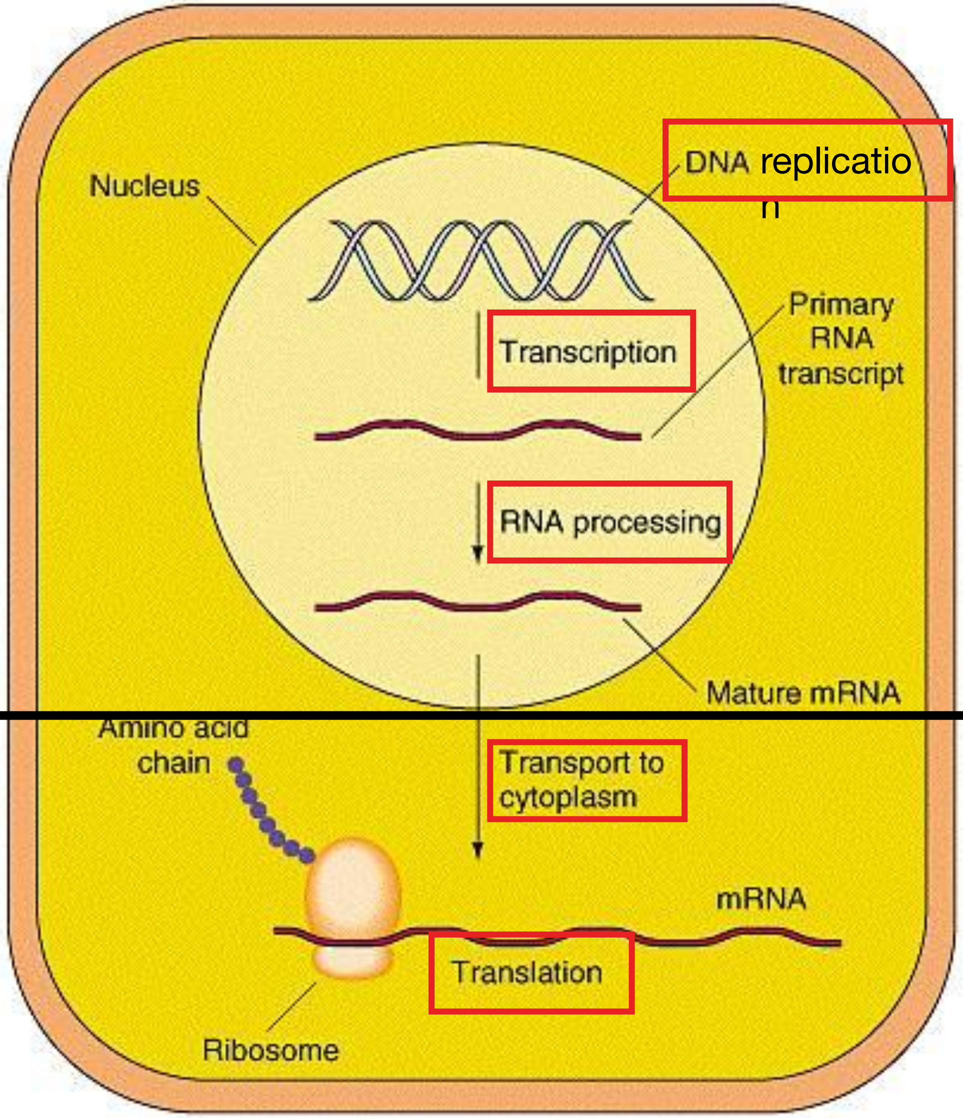
Most processes known to be controlled by RNAi include synthesis of dsRNA from DNA or RNA, cleavage of dsRNAs by distinct RNases into small RNAs, small RNAs then are involve in specific biological process.

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

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.

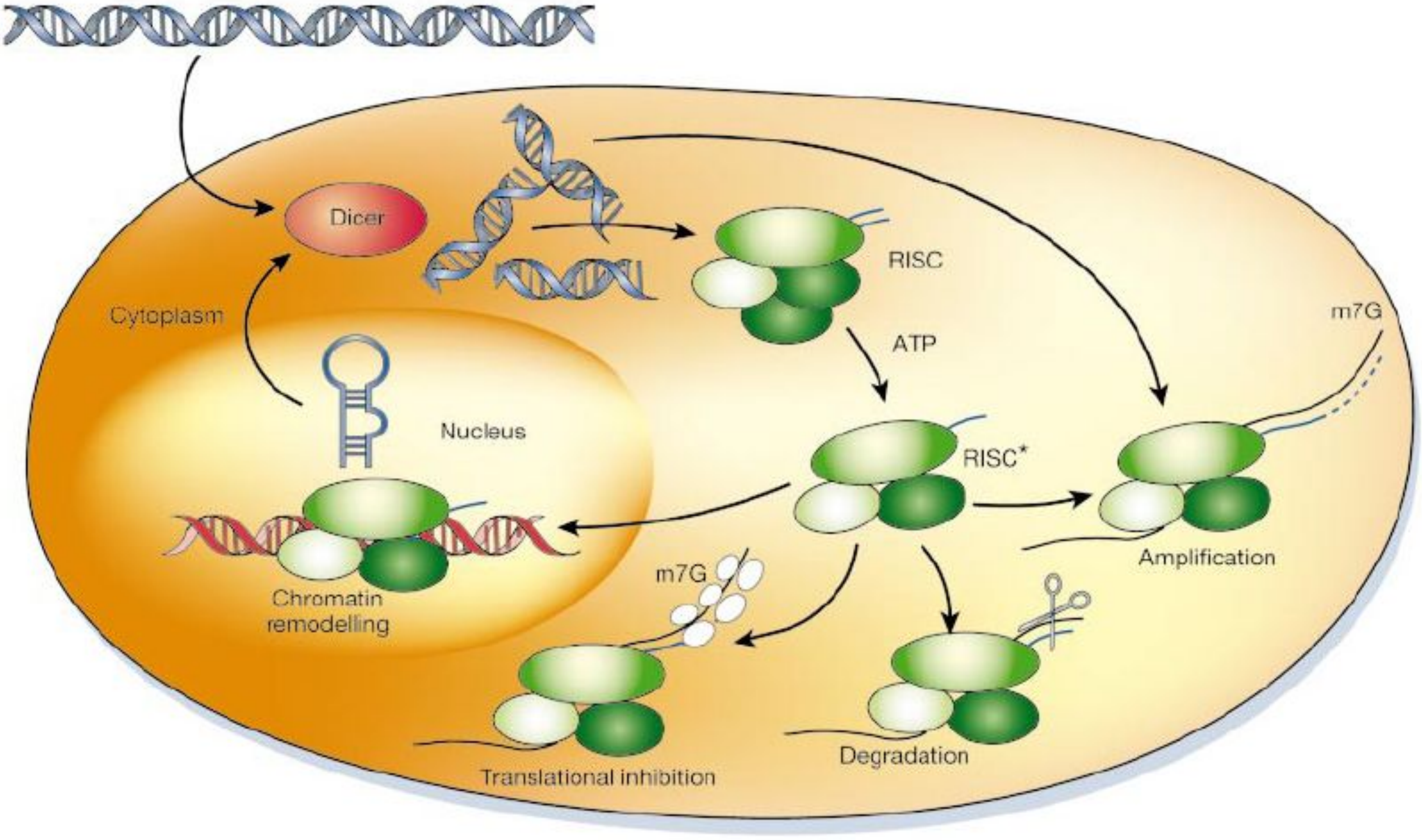
- These molecules act as trigger signals for the PTGS machinery, guiding the cell to an alert state-and the dsRNA molecules are destroyed

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

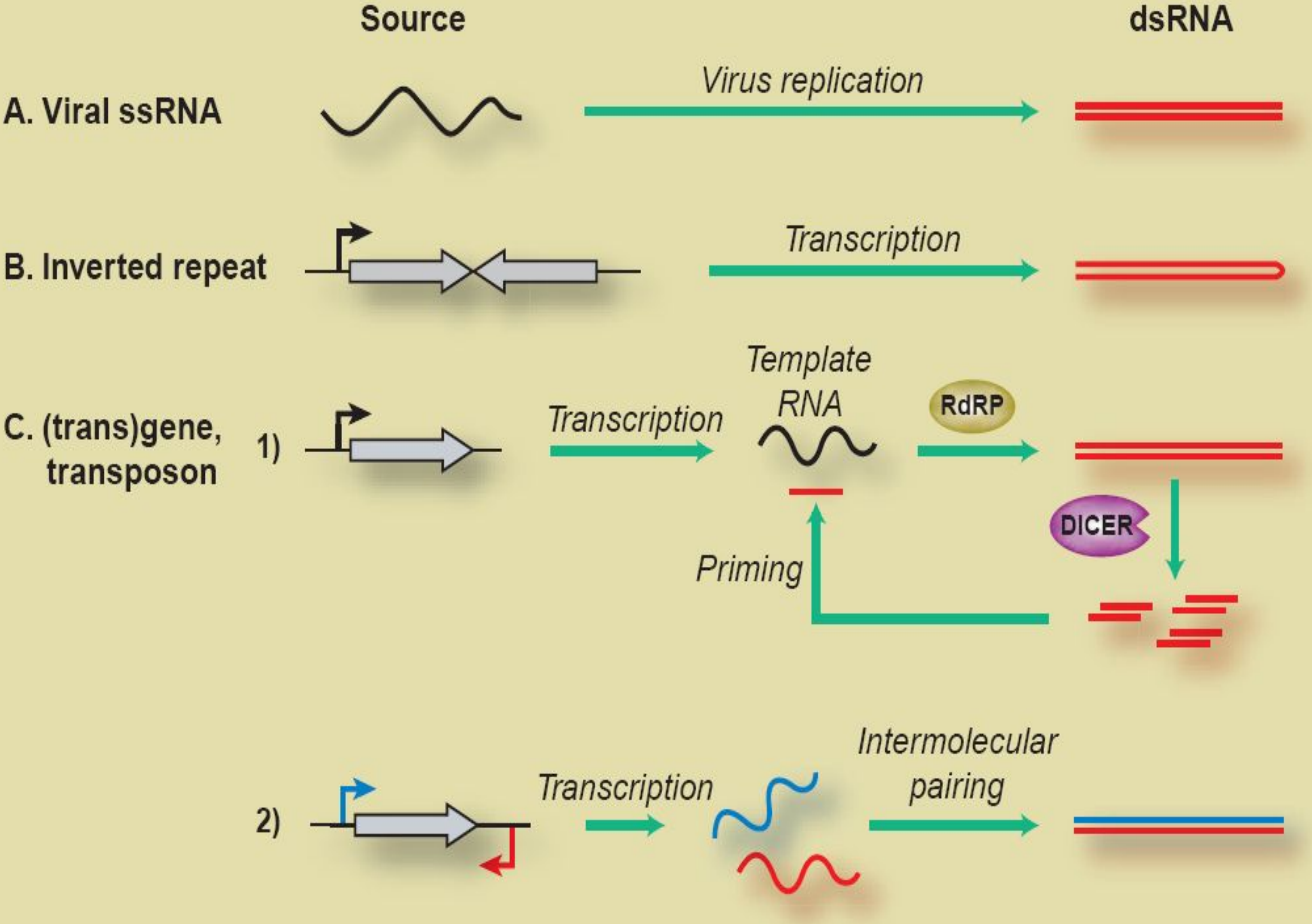
The effects of a dsRNA in a cell



Hannon G (2002). RNA interference. Nature 418: 244-251.

How did small RNA generate?

Exogenous or endogenous!



Source

dsRNA

A. Viral ssRNA

Virus replication

B. Inverted repeat

Transcription

C. (trans)gene,
transposon

1)

Transcription

Template
RNA

RdRP

DICER

Priming

2)

Transcription

Intermolecular
pairing

Now…….

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

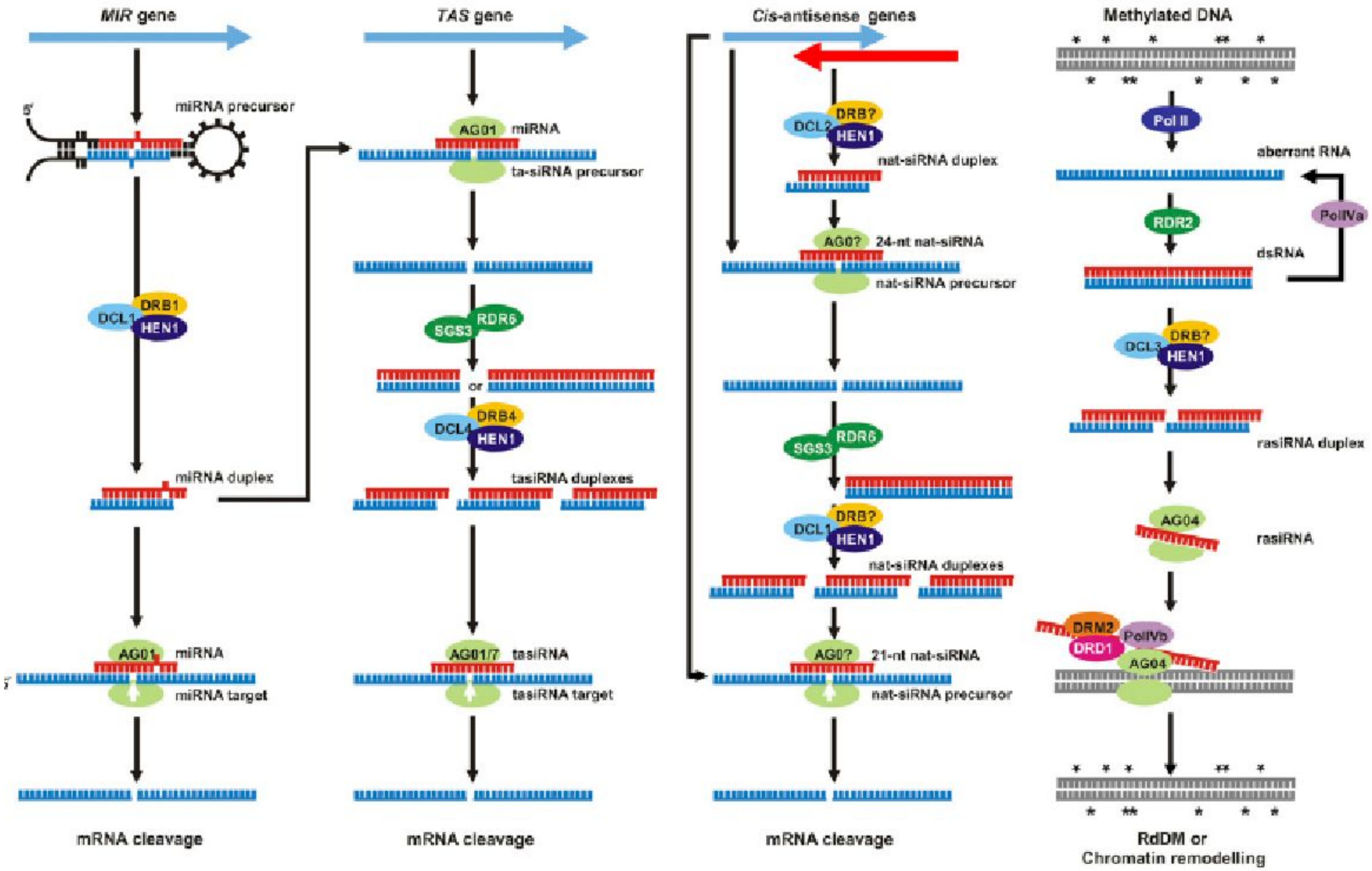


Figure 3. The parallel RNA silencing pathways of Arabidopsis. Schematic representation of the parallel DCL/sRNA-directed RNA silencing pathways in the model dicotyledonous species Arabidopsis, outlining the specific step or steps in each pathway for the individual RNA silencing-associated proteins mentioned 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 mico (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
S.cerevisae	0	0	0
S.pombe	1	1	1
C.elegans	1	27	4
D.melanogaster	2	5	0
A.thaliana	4	10	6
H.sapiens	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

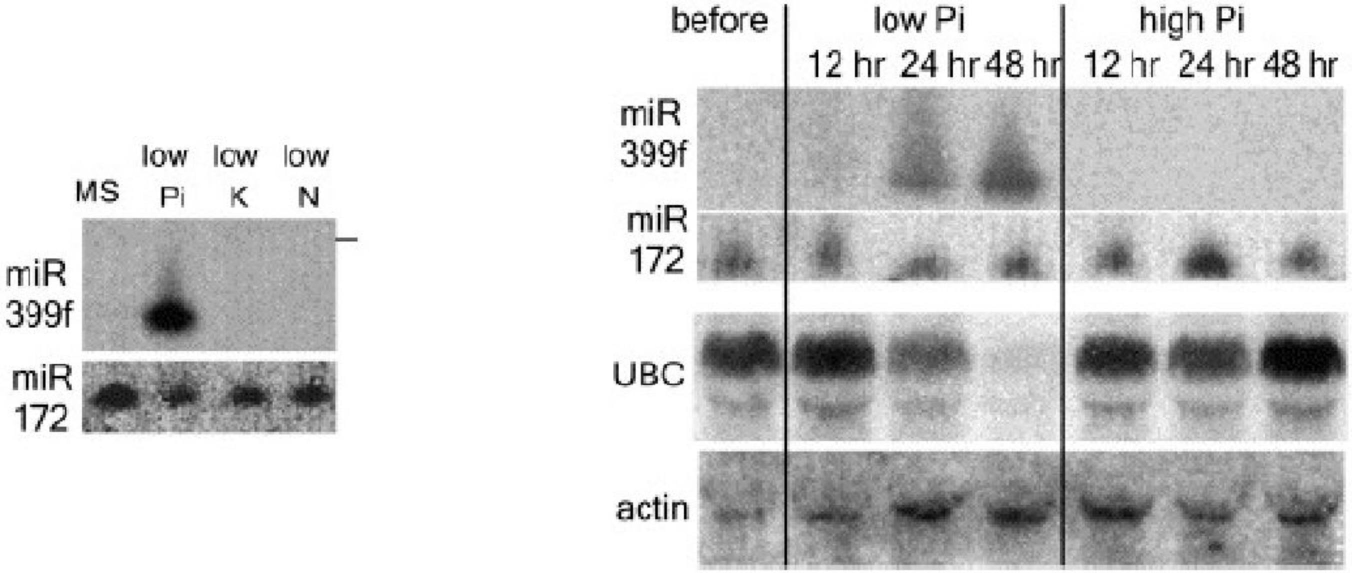
C. elegans
>100 miRNAs

D. melanogaster
>100 miRNAs

H. sapiens/
M. musculus
>200 miRNAs

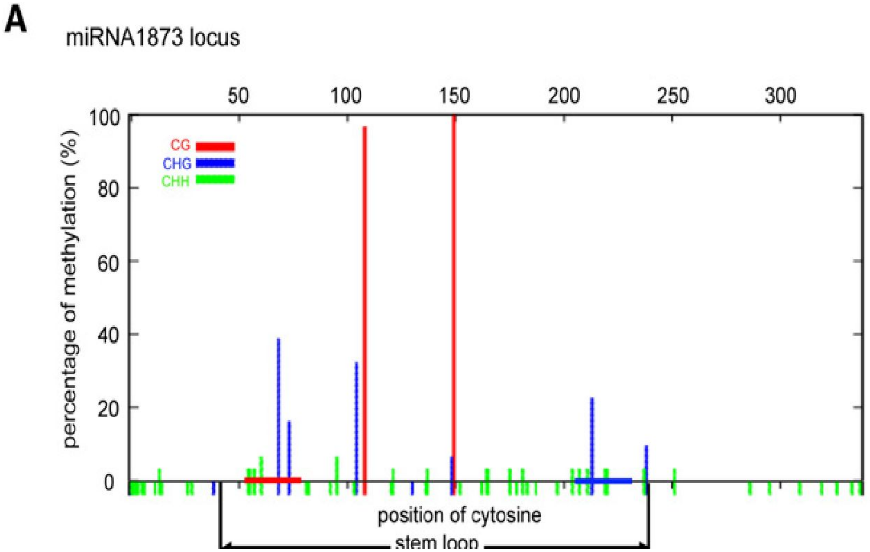
See the RNA Family database—http/www.sanger.ac.uk/software/rfam/

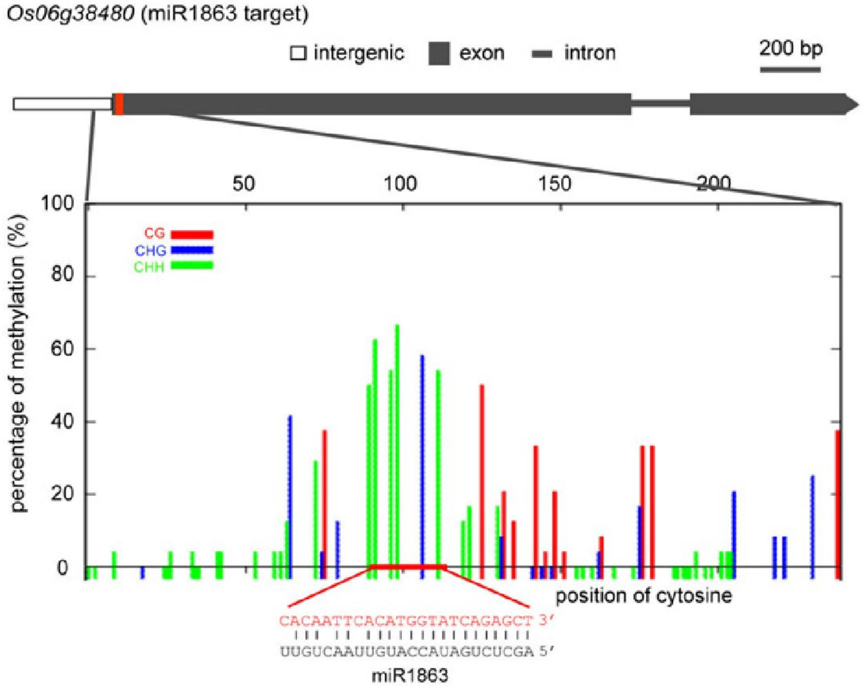
miRNA-controlled stress adaptation

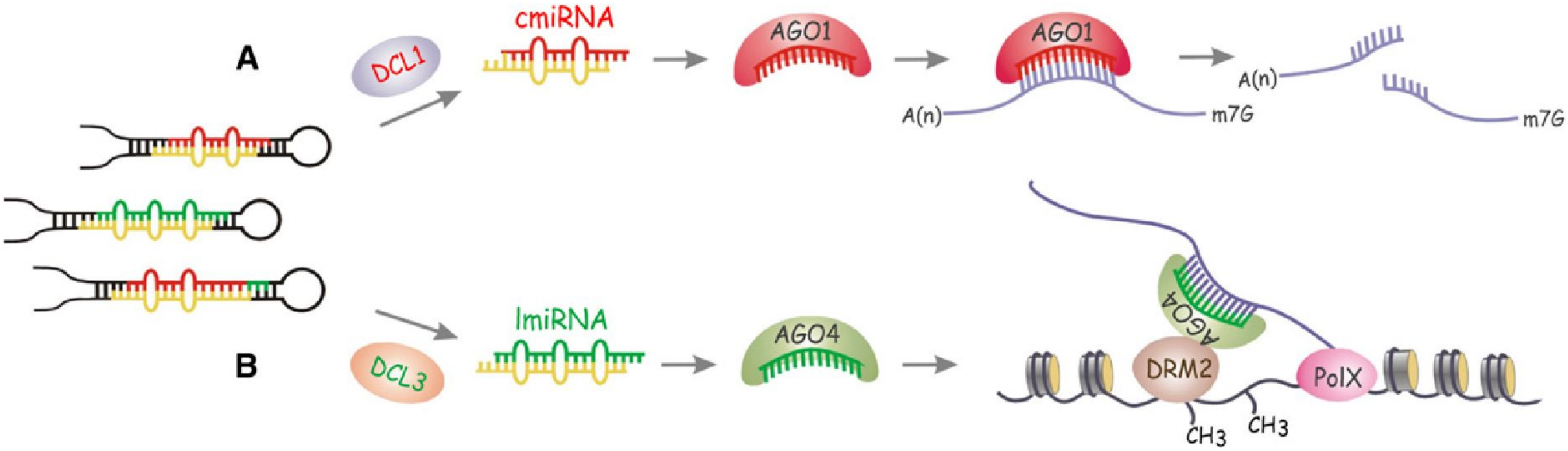


miR399 ⊣ UBC ⊗ AtPT1 → phosphate uptake

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



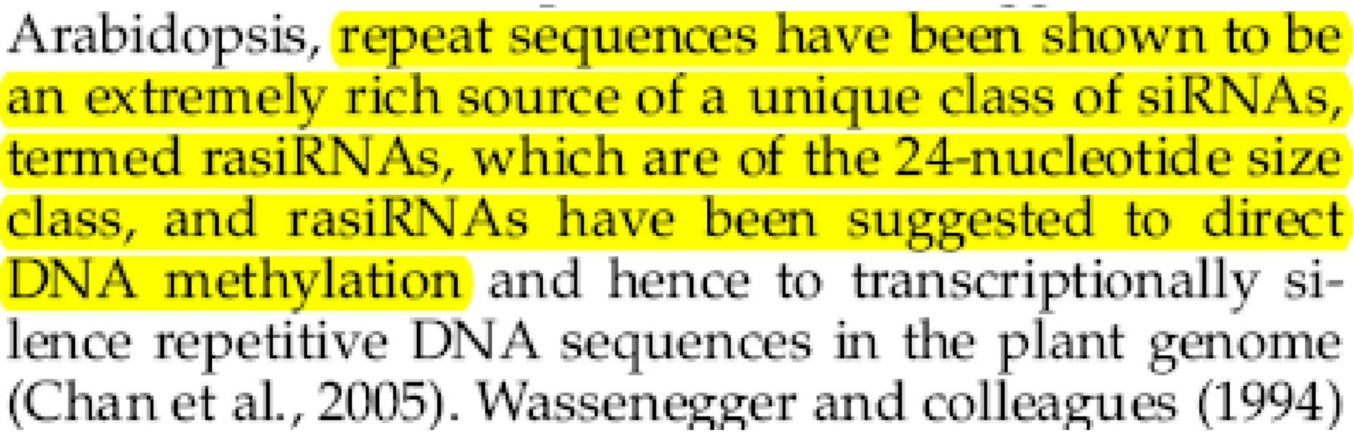




DNA Methylation Mediated by a MicroRNA Pathway, Yijun Qi et al, **Cell**, 2010, 14May

heterochromatic siRNAs
hc-siRNAs

rasiRNA



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

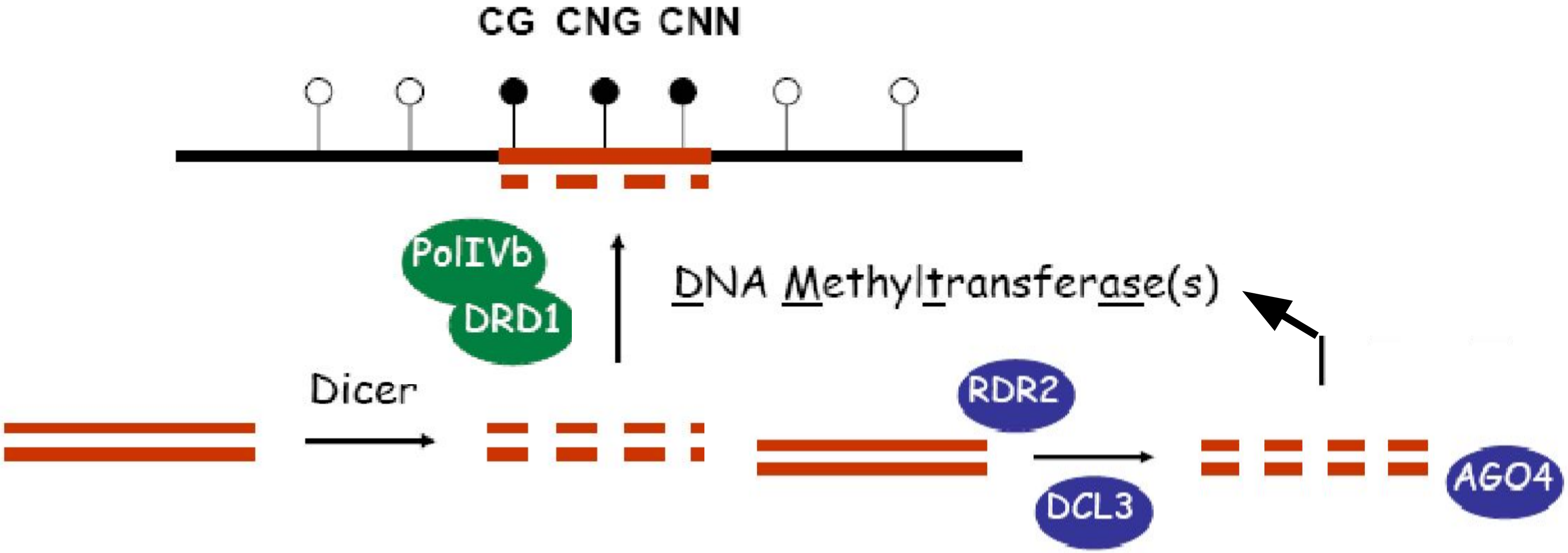
rasiRNA

- 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/^{m}C-pro

dsRNA can also interact with DNA and induce epigenetic changes…

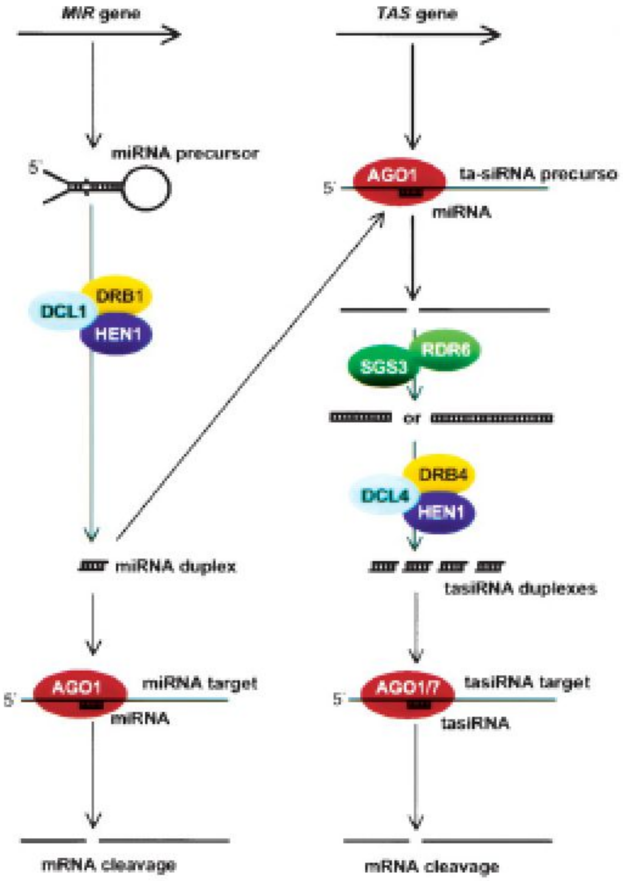
Epimutations:

- 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

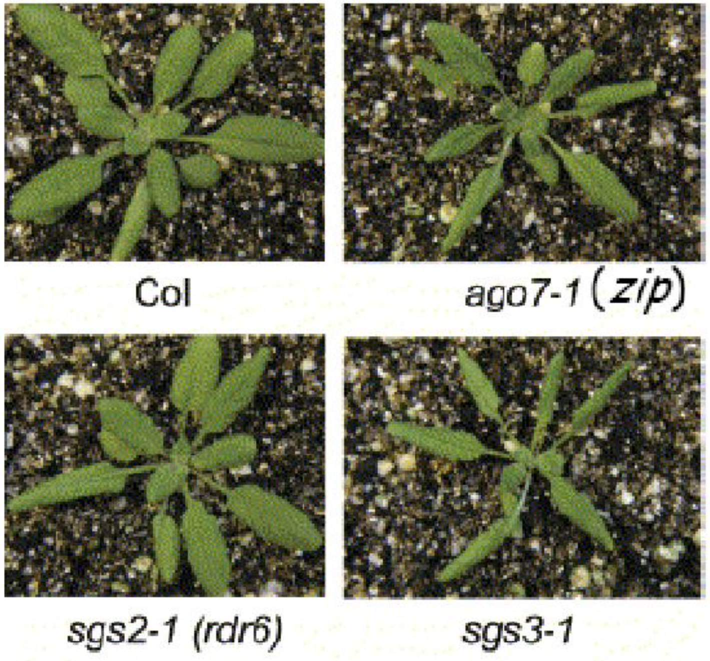
trans-acting siRNAs (ta-siRNAs)

trans-acting siRNAs (ta-siRNAs)



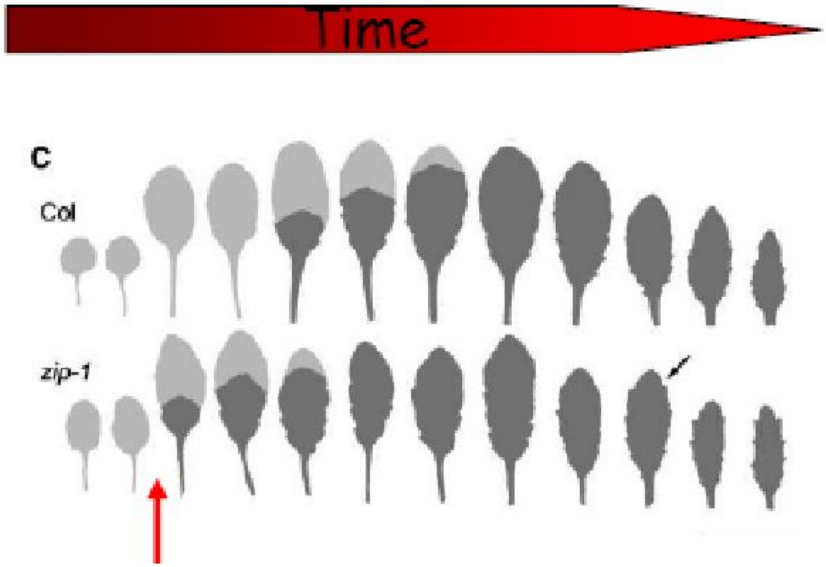
Vaucheret 2006, *Genes* Dev. 20, 759-771.

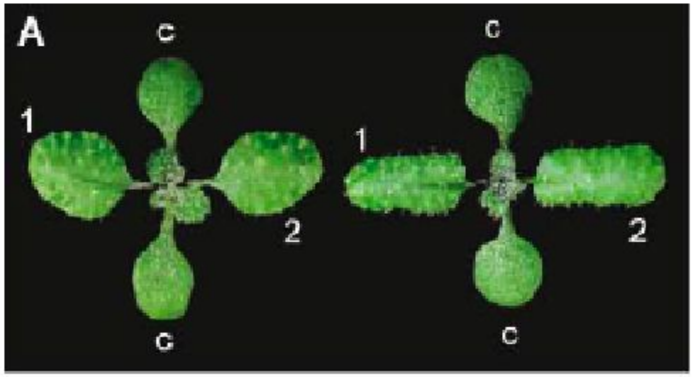
ta-siRNAs are involved in leaf development and polarity



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

Mutation of an Argonaute gene enhances phase change





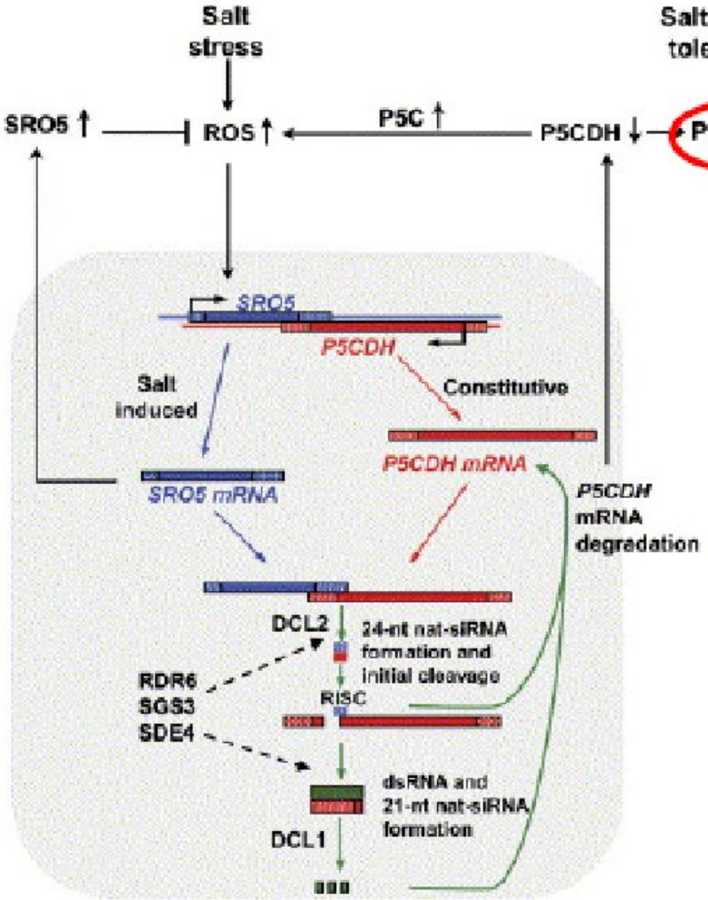
WT *zip-1*

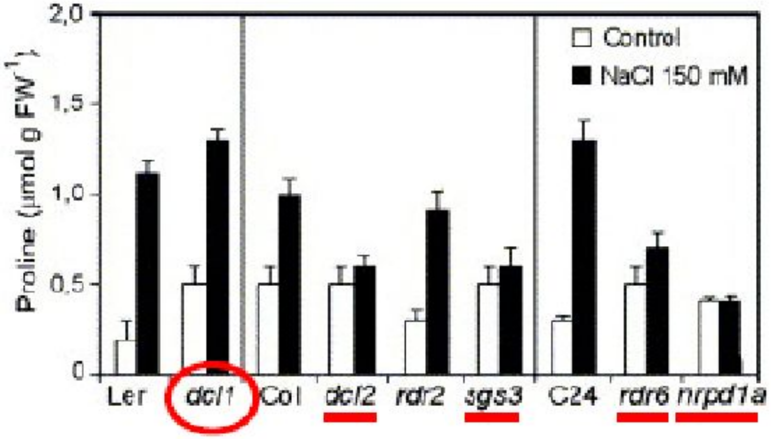
ZIP = *AGO 7*

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

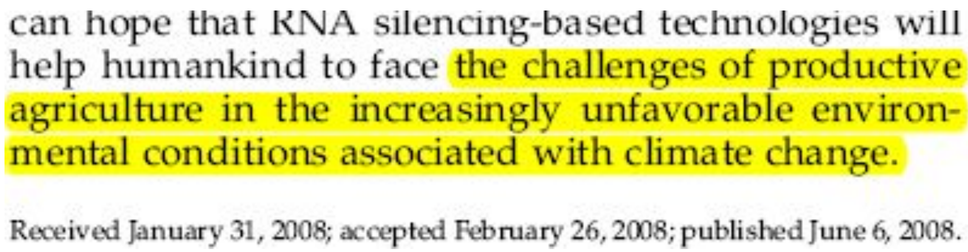
- RNA-RNA interaction in the cytoplasm
- RNAi regulates gene expression at the posttranscriptional level
- Regulation of mRNAs occurs via degradation and/or translational inhibition
- RNAi is conserved across kingdoms

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.

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Thank you for your attention