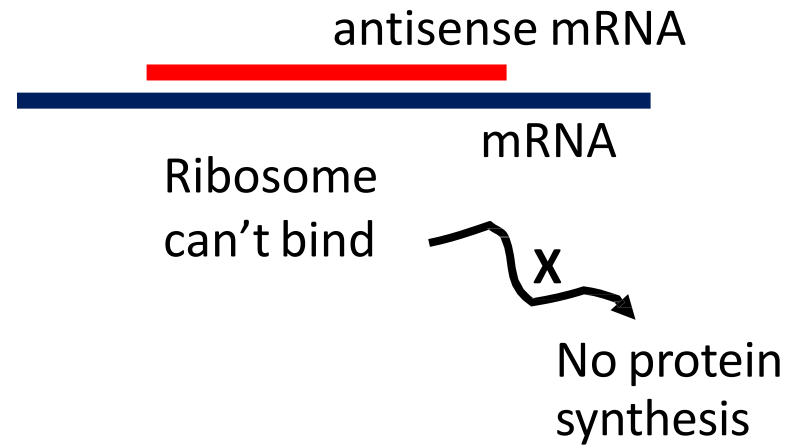
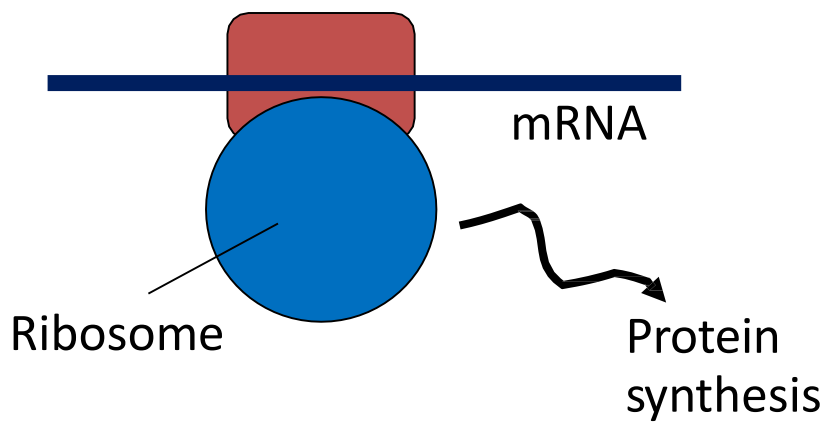


RNA Interference (RNAi)

Before RNAi...



Antisense RNA inhibits protein synthesis by blocking Translation

What is RNAi?

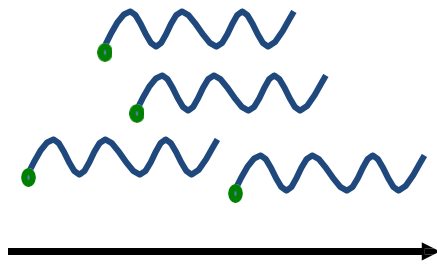
- **RNA interference (RNAi):** Cellular process by which an mRNA is targeted for degradation by a dsRNA with a strand complementary to a fragment of such mRNA.
- A selective gene **knock-down** phenomenon.

Specific terms for gene silencing

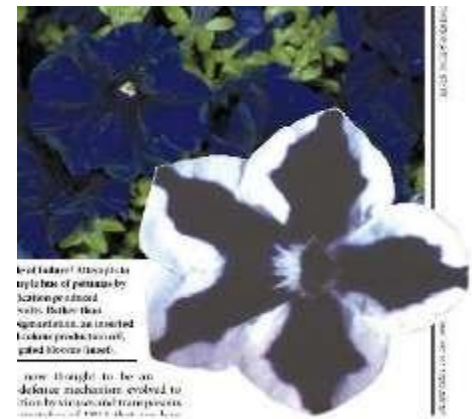
- ✓ Post-transcriptional gene silencing (**PTGS**) - Plants
- ✓ **Quelling** - Fungi
- ✓ RNA interference (**RNAi**) – Animals
- ❖ RNAi operates and its natural **role for virus defence** and **endogenous gene regulation** in plants

- ✓ RNAi phenomenon was discovered in transgenic plant *Petunia hybrida* L. (Napoli *et al.* 1990)
- ✓ They want to enhance anthocyanin pigments
- ✓ Unexpectedly, transgenic plants producing white or chimeric flowers were obtained instead of dark purple flowers due to the silencing of endogenous homologous gene and this phenomenon was termed as “**co-suppression**”.

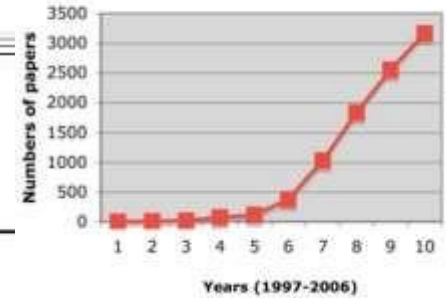
Petunia



Chalcone Synthase
(sense strand)



TIME LINE



Garcia-Blanco (2007)



RNAi in *Drosophila*, plants

SiRNA in mammalian cells

RNAi mechanism

1990

1992

1998

2001

2002

2003

2004

2005

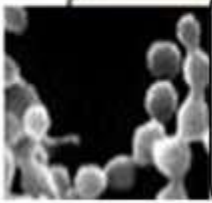
2006

Post transcriptional gene silencing (PTGS)

RNAi in *C. elegans*

Science named RNAi 'Technology of the year'

Nobel prize Fire & Mello



Quelling in *Neurospora*





The Nobel Prize in Physiology or Medicine 2006

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

Andrew Z. Fire
Craig C. Mello



Photo: L. Cicero/Stanford



Photo: R. Carlin/UMMAS

Andrew Z. Fire

🏆 1/2 of the prize

USA

Stanford University School
of Medicine
Stanford, CA, USA

Craig C. Mello

🏆 1/2 of the prize

USA

University of
Massachusetts Medical
School
Worcester, MA, USA

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[‡]

RNA silencing works on at least three different levels in plants *viz.*

1. Cytoplasmic silencing by **dsRNA** results in cleavage of mRNA
2. Endogenous mRNAs are silenced by micro-RNAs (**miRNAs**), which negatively regulate gene expression by base-pairing to specific mRNAs, resulting in either **RNA cleavage or blocking protein translation** called **PTGS**.
3. RNA silencing is associated with sequence-specific **methylation of DNA** and the consequent suppression of transcription called transcriptional gene silencing [**TGS**].

Multiple pathways for RNAi

There are two main RNAi pathways:

1. small interfering RNAs (**siRNAs**) generated via processing of **longer dsRNA** and
2. microRNAs (**miRNAs**) that are generated via processing of **stem loop precursors**

Main component of RNAi machinery for siRNA generation

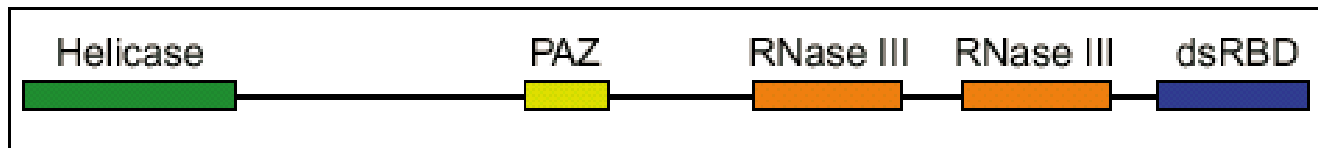
There are four component for siRNAs generation

1. Dicer
2. small Interfering RNA (siRNA)
3. RNA-Induced Silencing Complex (RISC)
4. RNA-Dependent RNA Polymerase (RdRP)

Dicer

- ✓ First discovered by Bernstein *et al.* (2001) in *Drosophila*.
- ✓ Enzyme belonging to the **RNase III ribonuclease** family.
- ✓ Cutting mechanism of the Dicer enzyme is ATP-independent (Kuznetsov 2003).
- ✓ Responsible for the processing of dsRNA into siRNAs
- ✓ Cleaves dsRNA or pre-miRNA-leaves 3' overhangs and 5' phosphate groups.
- ✓ Initiates RNAi.

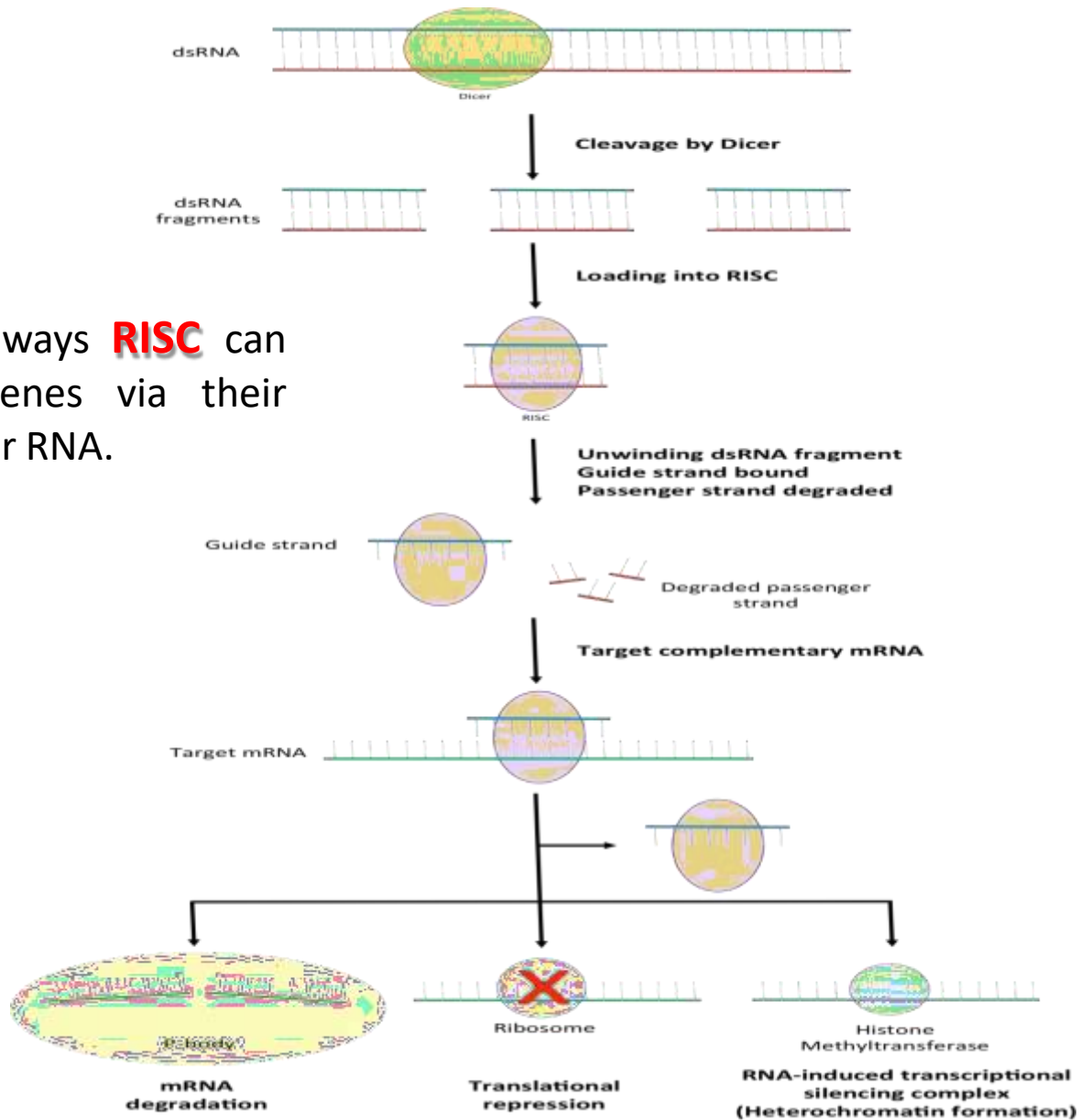
- **Functional domains in Dicer** (Bernstein *et al.*, 2001)
 - Helicase
 - PAZ domain
 - Tandem RNase-III domains
 - dsRNA binding domain
- **Dicer-like proteins in Plants**
 - DCL1 - miRNA generation and sRNA production from inverted repeats
 - DCL2 - creates siRNA from cis-acting antisense transcripts
 - DCL3 - generates siRNA which aids in chromatin modification
 - DCL4 - involved in trans-acting siRNA metabolism and transcript silencing at the post-transcriptional level.



RNA-Induced Silencing Complex (RISC)

- ✓ First discovered in *Drosophila*, by Hammond *et al.* (2000)
- ✓ Nuclease complex composed of proteins and siRNA
- ✓ Targets and destroys endogenous mRNAs complementary to the siRNA
- ✓ RISC consists of both protein and RNA
- ✓ RNAi effector complex
 - Critical for target mRNA degradation or translation inhibition
- ✓ Activities associated with RISC
 - Helicase
 - Endonuclease and exonuclease “Slicer”
 - “homology seeking”/RNA binding

Different ways **RISC** can silence genes via their messenger RNA.



RNA-dependent RNA Polymerase (RdRP)

- ✓ RdRP – Triggering and amplifying the silencing effect
- ✓ Systemic nature of RNAi
- ✓ RdRP activity found in plants and *C. elegans*
- ✓ May explain efficiency of RNAi

siRNA

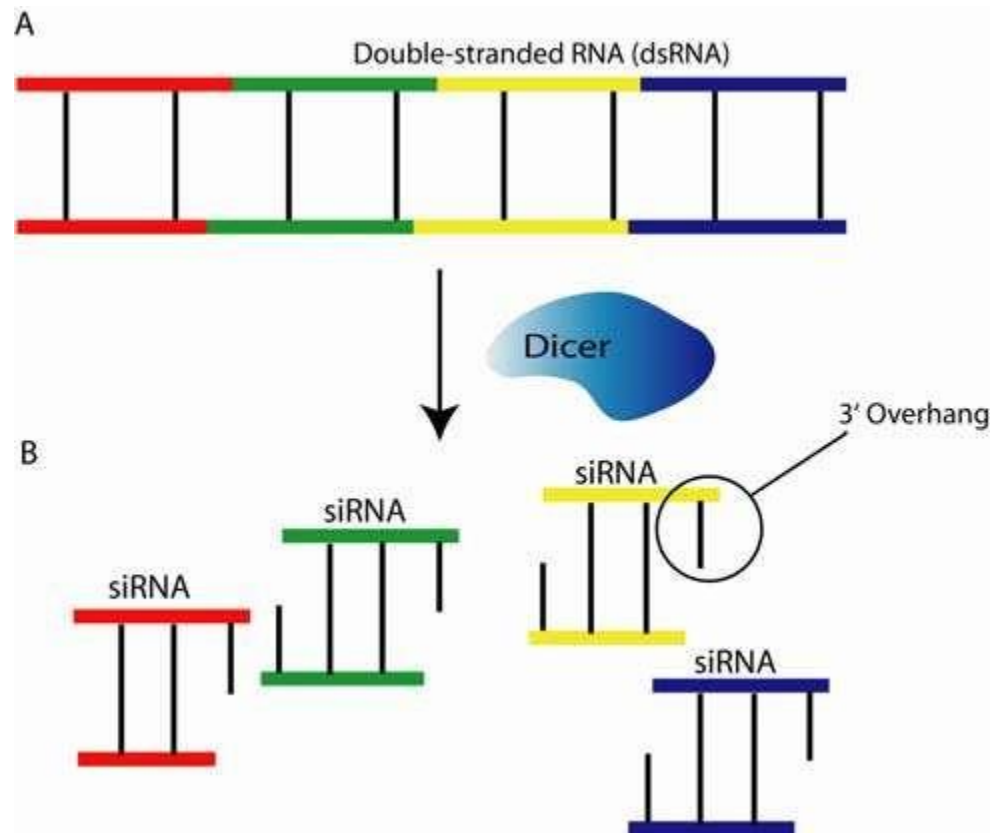
- ✓ 21-23 nucleotide dsRNA that mediate PTGS
- ✓ Produced *in vivo* by cleavage of dsRNA
- ✓ Amplification by an RNA-dependent RNA polymerase (RdRP) may occur
- ✓ Incorporated into the RISC guiding it to mRNA
- ✓ Complementary to a specific sequence of target mRNA for degradation.

Mechanism of RNAi

- Initiation phase
- Maintenance phase
- Signal amplification and spreading phase

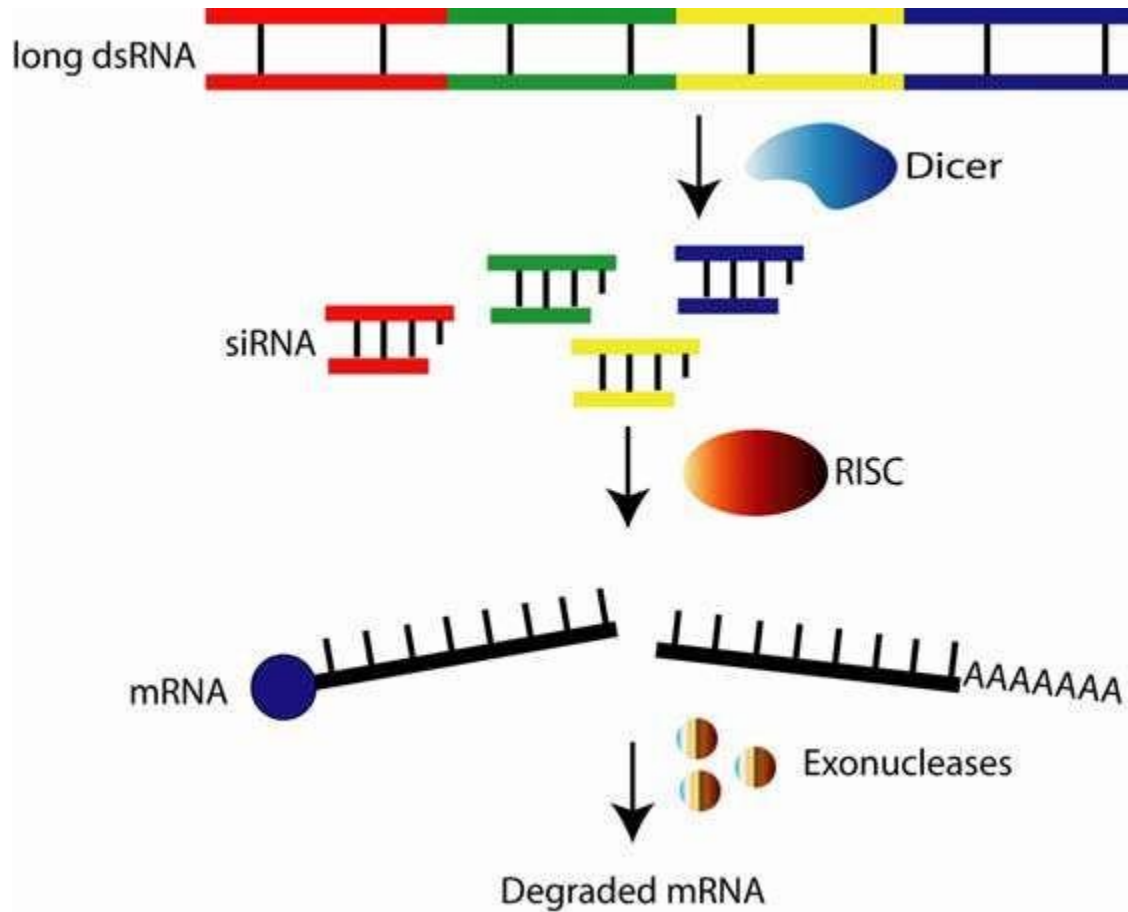
Initiation phase

dsRNA cut by Dicer

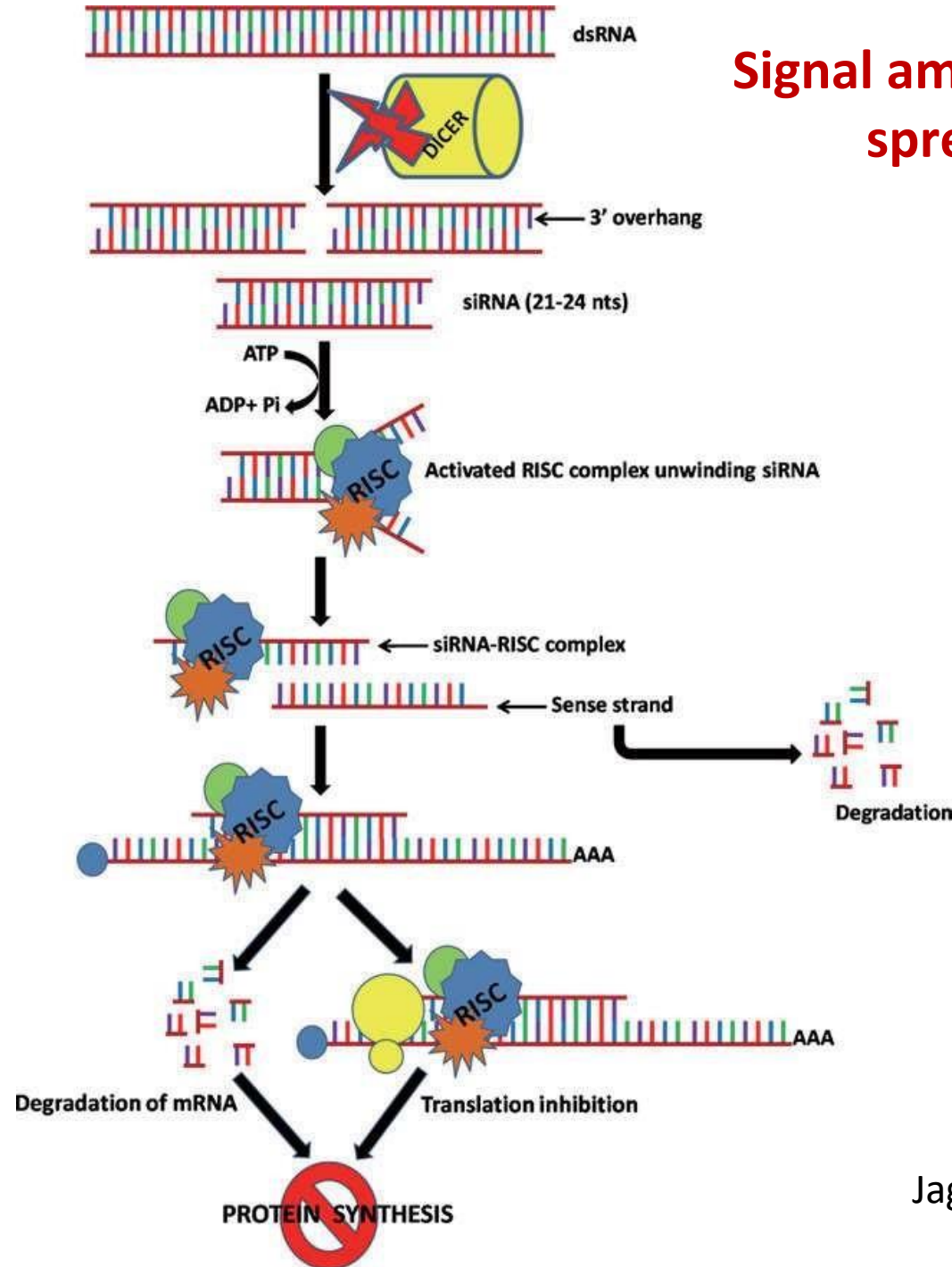


Maintenance phase

siRNA incorporated into RISC



Signal amplification and spreading phase



Cell to cell movement of RNA silencing.....

a. Short distance movement :

- Exogenous ds RNA is processed by Dicer like enzymes into 21nt and 24nt Primary SiRNAs.
- 24nt SiRNA was shown to be dispensable for local movement.
- 21nt SiRNA may spread over 10-15 adjacent cells in the absence of relay amplification.

b. Extensive silencing movement:

- ❖ Perception of 21nt Si RNAs in recipient cell triggers *de nova* synthesis of dsRNA
- ❖ The dsRNA further processed by dicer resulting in 21nt secondary siRNA
- ❖ This further Spread over 10-15 cells.

Main component of RNAi machinery for miRNA generation

There are six component for miRNAs generation

1. Drosha
2. Dicer
3. Argonaute (Ago)
4. small Interfering RNA (siRNA)
5. RNA-Induced Silencing Complex (RISC)
6. RNA-Dependent RNA Polymerase (RdRP)

Drosha

- ✓ Processes pri-miRNA into pre-miRNA
 - Leaves 3' overhangs on pre-miRNA
- ✓ Nuclear RNase-III enzyme [Lee et al., 2003]
 - Tandem RNase-III domains
- ✓ How does it identify pri-miRNA?
 - Hairpin terminal loop size
 - Stem structure
 - Hairpin flanking sequences
- ✓ Not yet found in plants
 - Maybe Dicer does its job?

Drosha

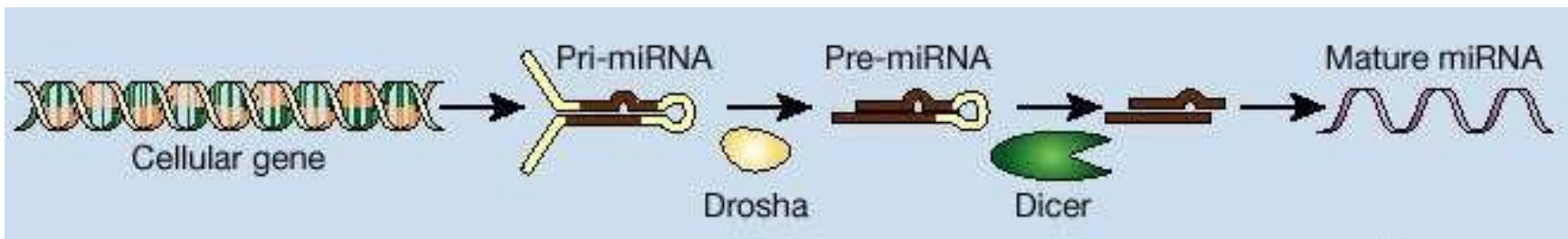


Argonaute (Ago)

- ✓ Consistently co-purifies with RISC [Hammond *et al.*, 2001]
- ✓ “Homology seeking” activity?
 - Binds siRNA and miRNA [Ekwall, 2004]
 - Distinguishes antisense strand [Novina and Sharp, 2004]
- ✓ Multiple Ago family proteins
 - Different RISCs
 - Tissue specific, Developmentally regulated
- ✓ Evidence for different RISCs [Tijsterman *et al.*, 2004]
 - Drosophila Dicer1 vs Dicer2/R2D2
 - Inhibition vs. degradation [Lee *et al.*, 2004]

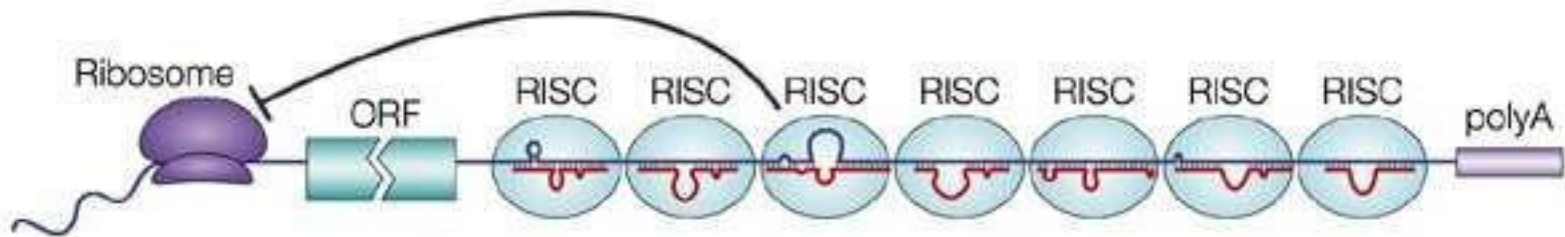
miRNA Biogenesis

- ✓ Transcribed from endogenous gene as pri-miRNA
 - Primary miRNA: long with multiple hairpins
 - Imperfect internal sequence complementarity
- ✓ Cleaved by Drosha into pre-miRNA
 - Precursor miRNA: ~70nt imperfect hairpins
 - Exported from nucleus
- ✓ Cleaved by Dicer into mature miRNA
 - 21-25nt
 - Symmetric 2nt 3' overhangs, 5' phosphate groups

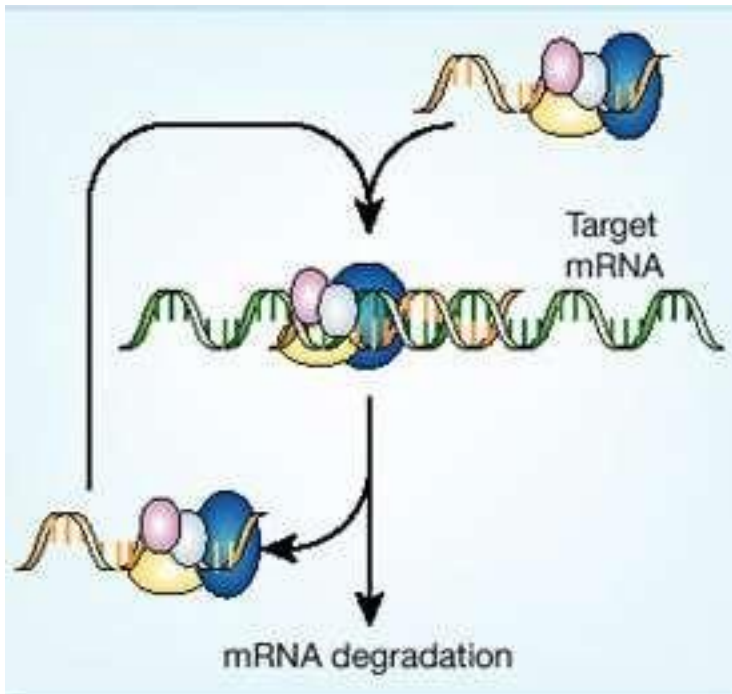


Translational Inhibition

- ✓ Imperfect match between siRNA or miRNA in RISC and target mRNA
- ✓ RISC usually binds 3' UTR
- ✓ Mechanism of inhibition... ????



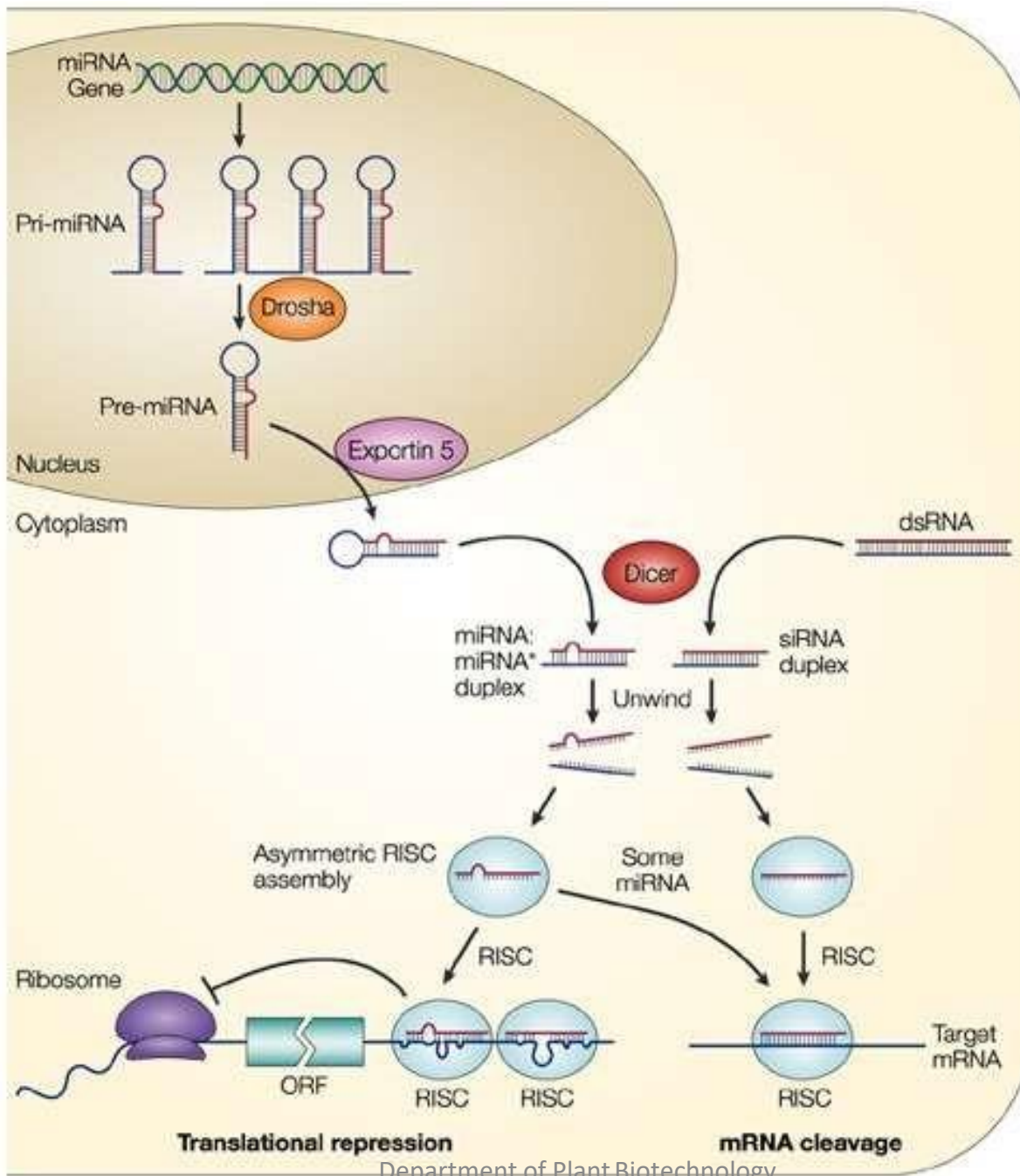
mRNA Degradation



- Perfect complementarity between siRNA or miRNA in RISC and the target mRNA
- Cleavage by RISC Slicer activity
 - Could be Dicer
 - Other endo/exonucleases
 - Recruitment of other components?

RNAi: Two Phase Process

- ✓ Initiation
 - Generation of mature siRNA or miRNA
- ✓ Execution
 - Silencing of target gene
 - Degradation or inhibition of translation



Initiation

Execution

Application of RNAi

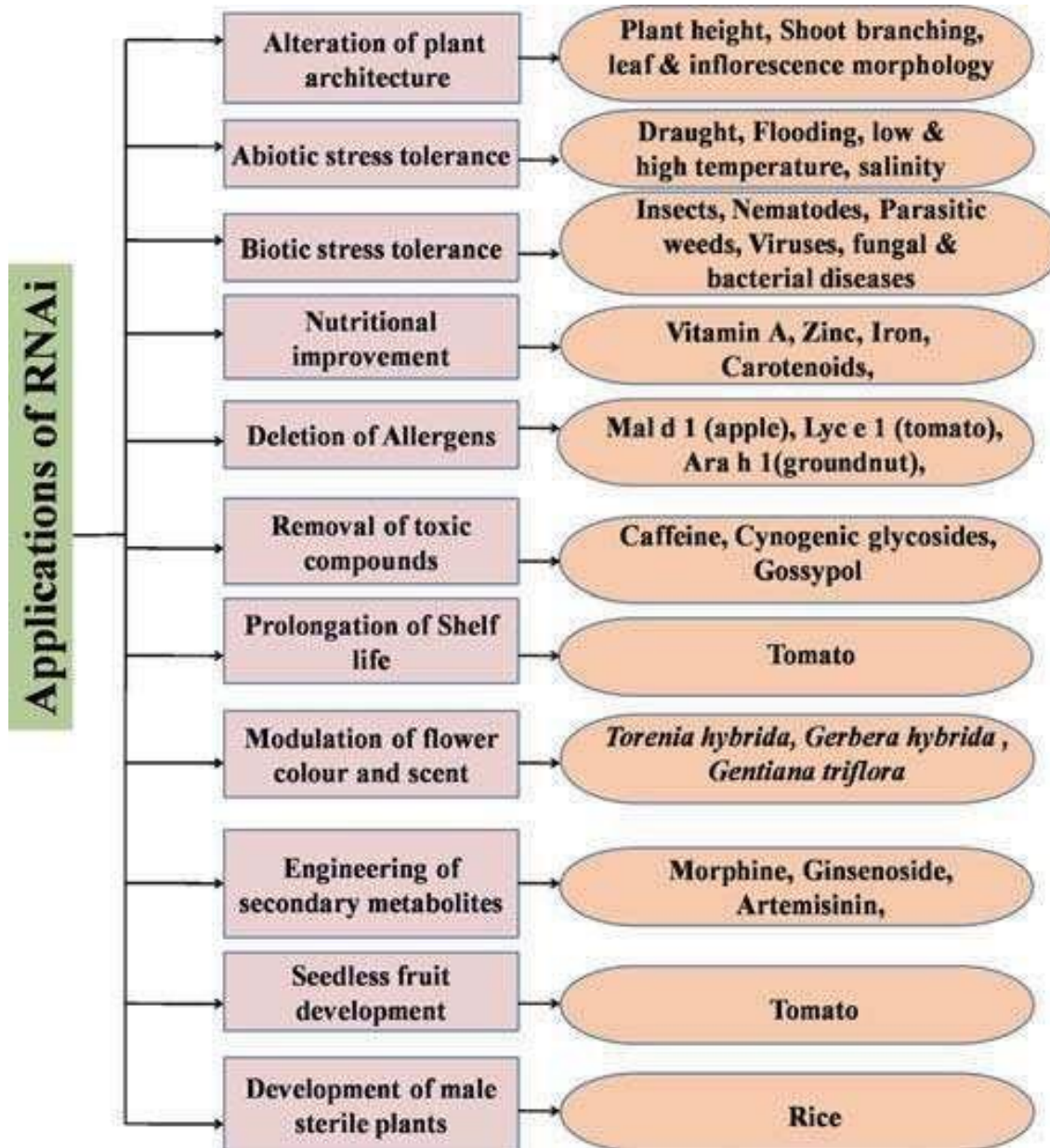


Table 1 A summary of commercial developments in agbiotech developed with RNA-mediated gene suppression.

Company	Crop	Trait gene	RNA-based gene suppression approach	Regulatory approval (animal feed, human food and/or environmental)	Phenotypic description
Calgene (now Monsanto)	Tomato (FLAVR SAVR)	Polygalacturonase	Antisense	US, Canada, Mexico, Japan	Delayed fruit ripening
Zeneca London, UK	Tomato	Polygalacturonase	Antisense and co-suppression	US, Canada, Mexico	Delayed fruit ripening
DNA Plant Technology	Tomato	Aminocyclopropane cyclase	Co-suppression	US, Canada, Mexico	Delayed fruit ripening
Vector Tobacco Durham, NC	Tobacco	Quinolinic acid phosphoribosyltransferase	Antisense	US	Reduced nicotine levels
DuPont Canada Agricultural Products Ontario, Canada	Soybean	Fatty acid desaturase	Co-suppression	US, Canada, Japan, Australia	High oleic acid soybean
Florigene Pty. Ltd.	Carnation	1-aminocyclopropane-1-carboxylic acid	Co-suppression	Australia, European Union	Longer vase life
US Department of Agriculture	Plum	Plum pox virus coat protein	Co-suppression	US	Viral resistance

Data source: Agbios (<http://www.agbios.com>)

Examples of novel plant traits engineered through RNAi.

Trait	Target Gene	Host	Application
Enhanced nutrient content	Lyc	Tomato	Increased concentration of lycopene (carotenoid antioxidant)
	DET1	Tomato	Higher flavonoid and bcarotene contents
	SBEII	Wheat, Sweet potato, Maize	Increased levels of amylose for glycemic anagement and digestive health
	FAD2	Canola, Peanut, Cotton	Increased oleic acid content
	SAD1	Cotton	Increased stearic acid content
	ZLKR/SDH	Maize	Lysinefortified maize
Reduced production of lachrymatory factor synthase	lachrymatory factor synthase gene	Onion	“Tearless” onion

<https://www.isaaa.org/resources/publications/pocketk/34/default.asp>

Reduced alkaloid production	CaMXMT1	Coffee	Decaffeinated coffee
	COR	Opium poppy	Production of nonnarcotic alkaloid, instead of morphine
	CYP82E4	Tobacco	Reduced levels of the carcinogen nornicotine in cured leaves
Heavy metal accumulation	ACR2	<i>Arabidopsis</i>	Arsenic hyperaccumulation for phytoremediation
Reduced polyphenol production	scadinene synthase gene	Cotton	Lower gossypol levels in cottonseeds, for safe consumption
Ethylene sensitivity	LeETR4	Tomato	Early ripening tomatoes
	ACC oxidase gene	Tomato	Longer shelf life because of slow ripening
Reduced allergenicity	Arah2	Peanut	Allergen free peanuts
	Lolp1, Lolp2	Ryegrass	Hypo-allergenic ryegrass

<https://www.isaaa.org/resources/publications/pocketk/34/default.asp>

Research focus

- ✓ **Sprayable RNAi technology** to control fusarium disease in barley were published by BASF
- ✓ **BioDirect** is the brand name coined by Monsanto for its sprayable RNAi platform.

RESEARCH ARTICLE

An RNAi-Based Control of *Fusarium graminearum* Infections Through Spraying of Long dsRNAs Involves a Plant Passage and Is Controlled by the Fungal Silencing Machinery

Aline Koch¹, Dagmar Bledenkopf¹, Alexandra Furch², Lennart Weber³, Oliver Rossbach⁴, Eitayb Abdellatef¹, Lukas Linicus¹, Jan Johannsmeier¹, Lukas Jelonek⁵, Alexander Goesmann⁶, Vinitha Cardoza⁶, John McMillan⁶, Tobias Mentzel⁷, Karl-Heinz Kogel^{1*}

1 Institute for Phytopathology, Centre for BioSystems, Land Use and Nutrition, Justus Liebig University, Giessen, Germany, **2** Institute of General Botany and Plant Physiology, Friedrich-Schiller-University, Jena, Germany, **3** Institute for Microbiology and Molecular Biology, Centre for BioSystems, Land Use and Nutrition, Justus Liebig University, Giessen, Germany, **4** Institute of Biochemistry, Centre for BioSystems, Land Use and Nutrition, Justus Liebig University, Giessen, Germany, **5** Institute for Bioinformatics and Systems Biology, Centre for BioSystems, Land Use and Nutrition, Justus Liebig University, Giessen, Germany, **6** BASF Plant Science LP, Research Triangle Park, Durham, North Carolina, United States of America, **7** BASF SE, Limburgerhof, Germany.



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OPEN ACCESS

Using the agronomically important **barley-*Fusarium graminearum* pathosystem**, demonstrated that a **spray application of a long noncoding dsRNA** (791 nt CYP3-dsRNA), which targets the three fungal cytochrome P450 *lanosterol C-14 α -demethylases*, required for biosynthesis of fungal ergosterol, inhibits fungal growth in the directly sprayed (local) as well as the non-sprayed (distal) parts of detached leaves.

Unexpectedly, efficient spray-induced control of fungal infections in the distal tissue involved passage of **CYP3-dsRNA via the plant vascular system** and processing into small interfering (si)RNAs by fungal DICER-LIKE 1 (*FgDCL-1*) after uptake by the pathogen.

3 fungal genes - CYP51B, CYP51A, CYP51C

Results

Fig . (A-C) Spray-induced gene silencing (SIGS) of *GFP* expression in *Fusarium graminearum* strain *Fg-IFA65GFP*

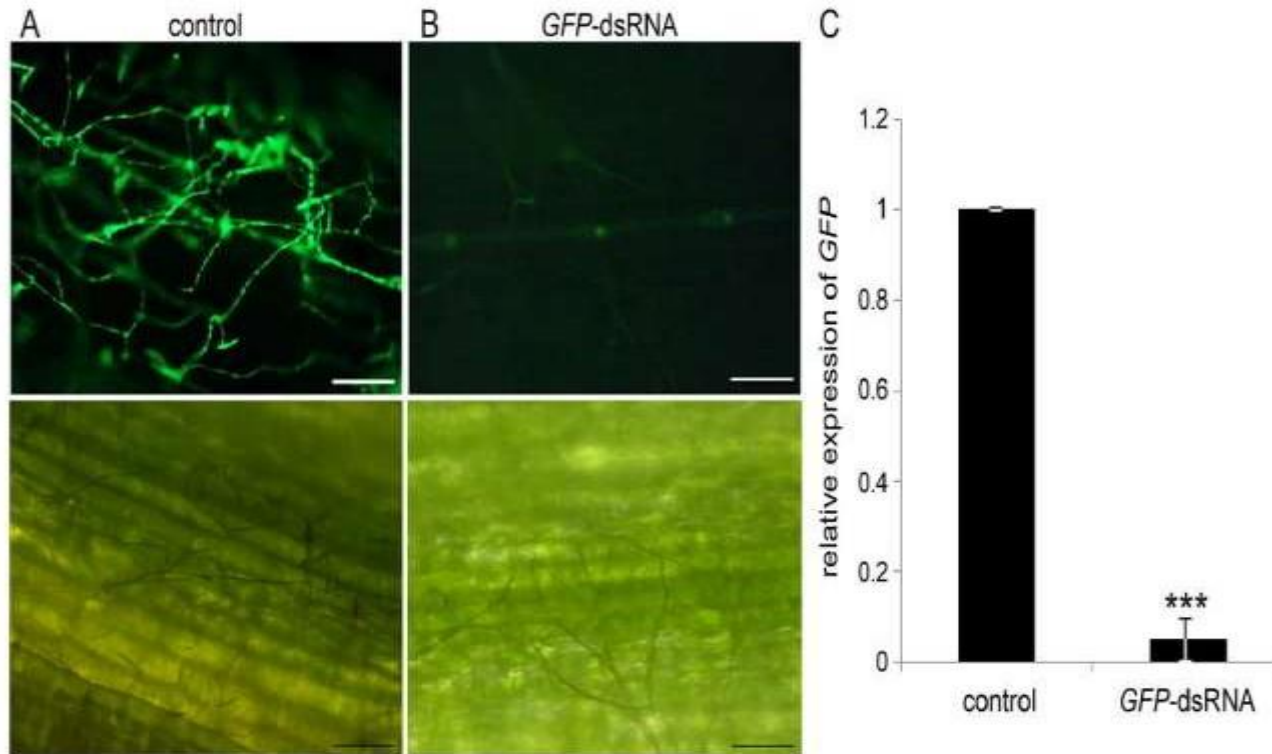
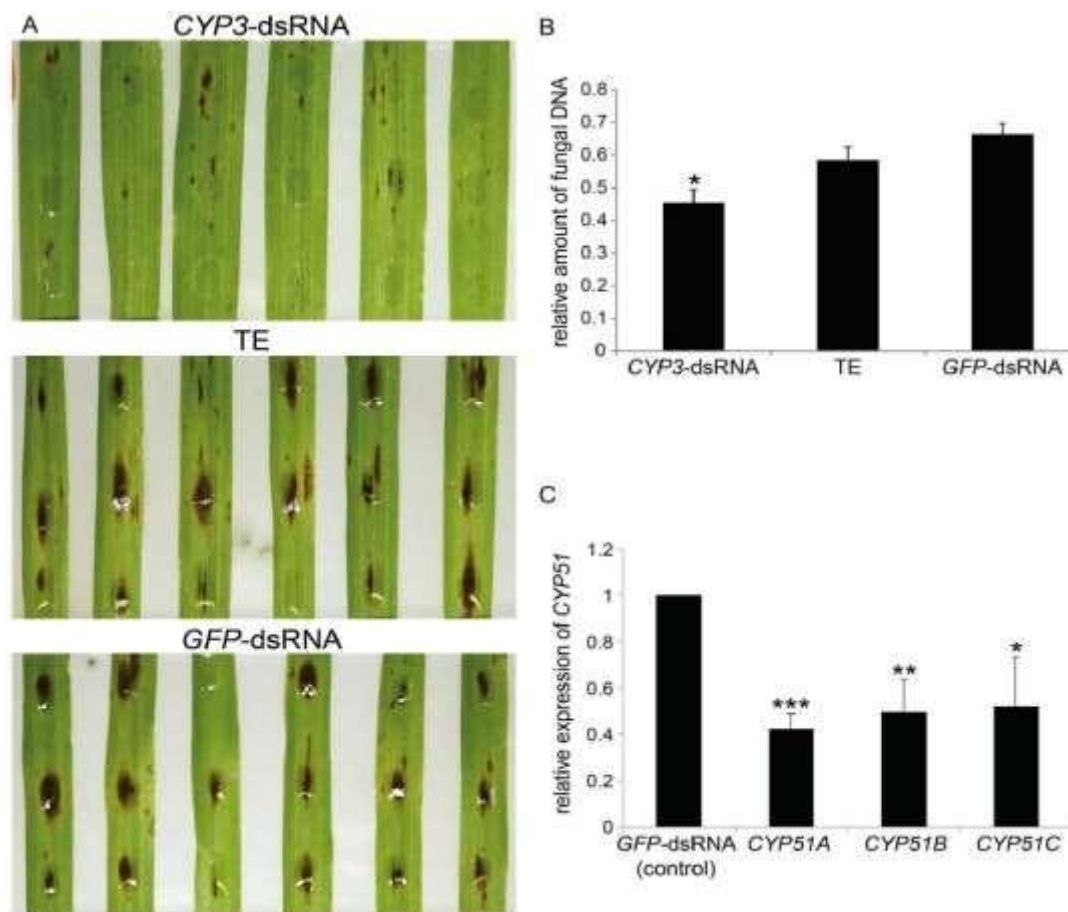
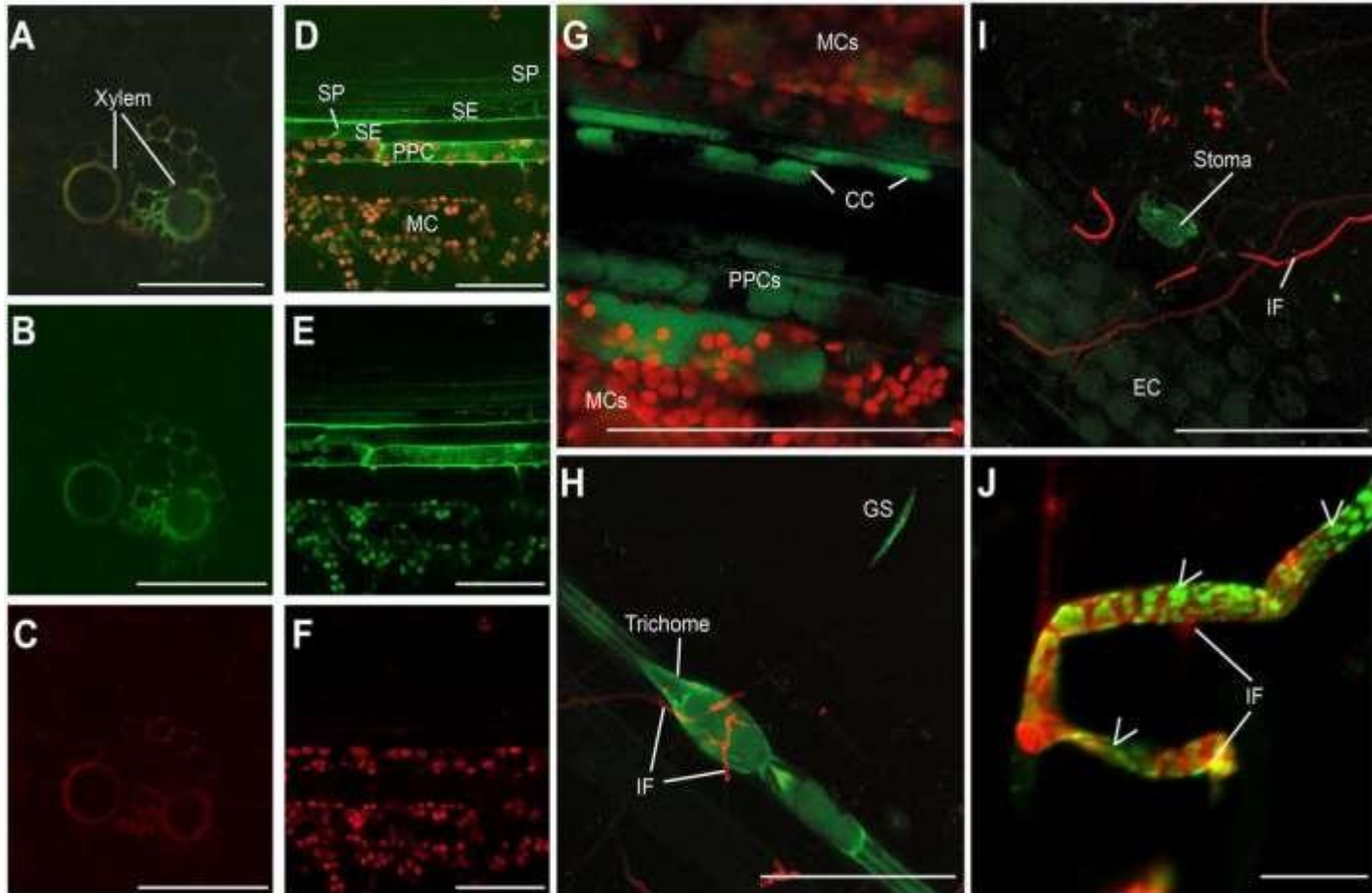


Fig . (A-C) SIGS-mediated control of *F. graminearum* on leaves sprayed with *CYP3-dsRNA*.



Uptake and transport of sprayed *CYP3-dsRNA*, it was labeled with the **green fluorescent dye ATTO 488 (CYP3-dsRNAA488)** and sprayed onto barley leaves.



SE, sieve element; CC, companion cell; SP, sieve plate; PPC, phloem parenchyma cell; MC, mesophyll cell, germinating spores (GS)

Possible pathways of host-induced gene silencing (**HIGS**) and spray-induced gene silencing (**SIGS**)

Review



Received: 30 June 2017

Revised: 11 September 2017

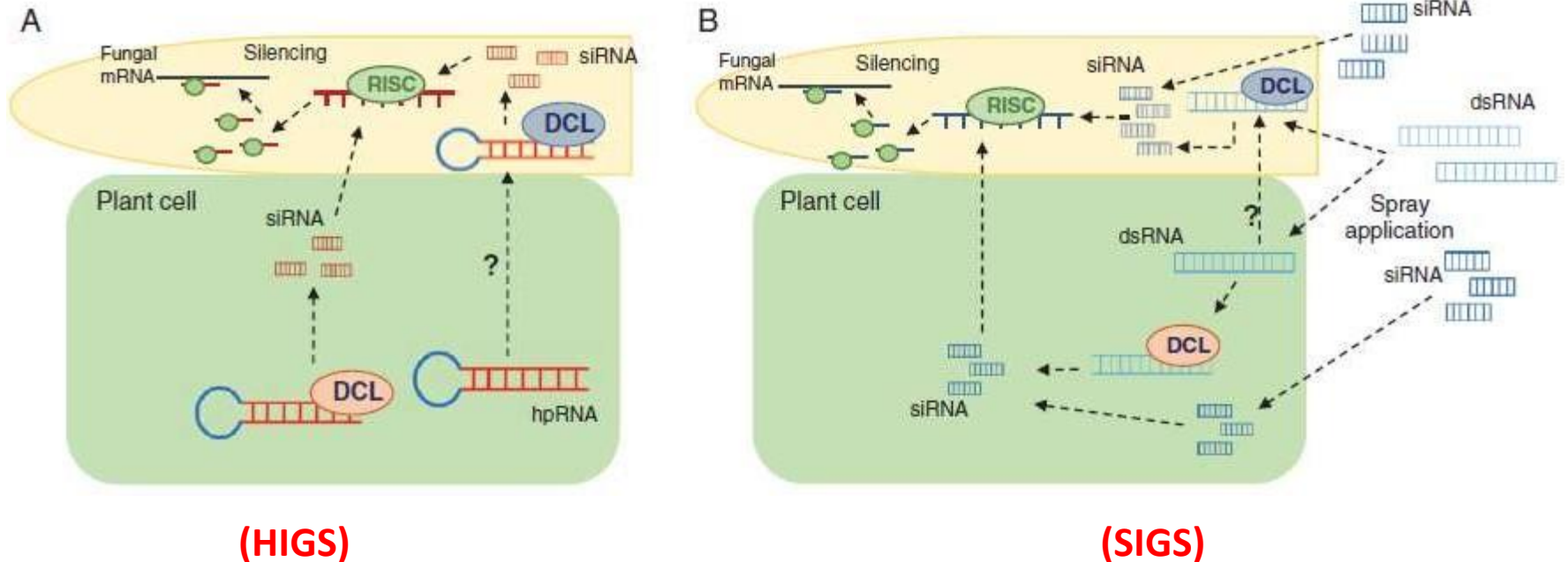
Accepted article published: 2 October 2017

Published online in Wiley Online Library: 22 November 2017

(wileyonlinelibrary.com) DOI 10.1002/ps.4748

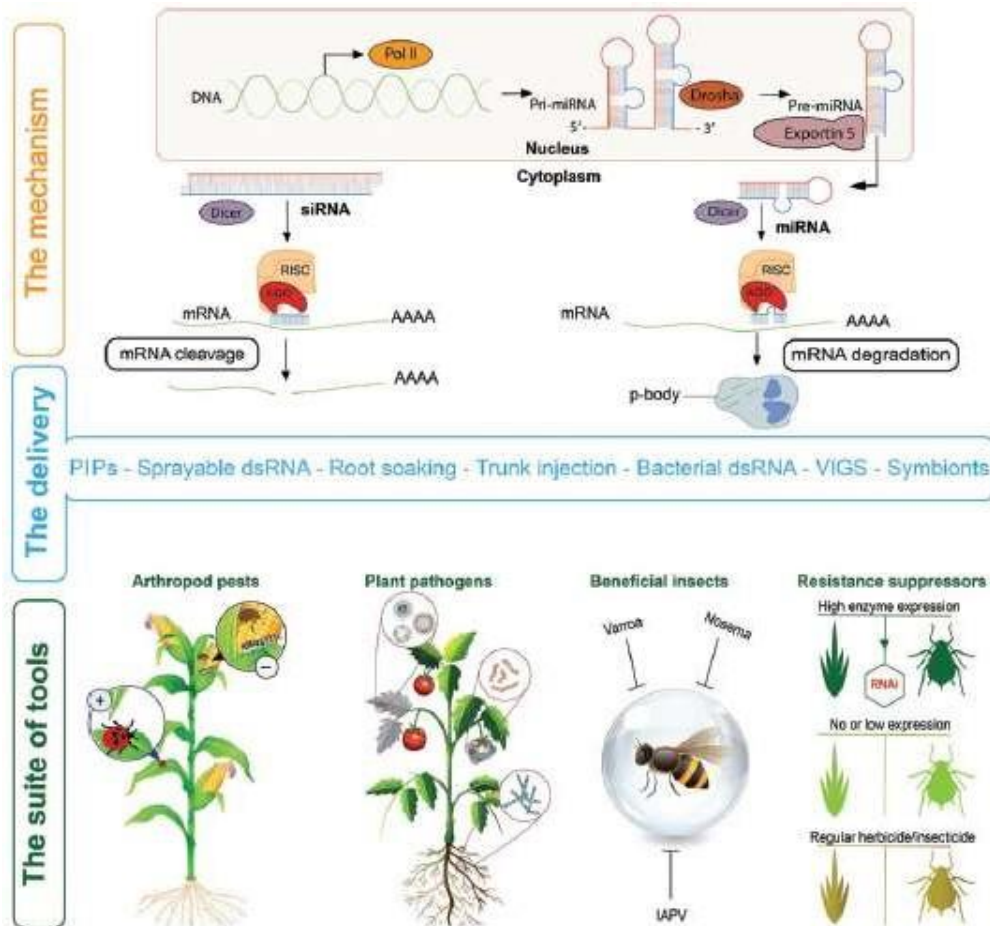
RNAi as an emerging approach to control *Fusarium* head blight disease and mycotoxin contamination in cereals

Ana Karla Machado,^a Neil A Brown,^{a,b} Martin Urban,^a
Kostya Kanyuka^a and Kim E Hammond-Kosack^{a*}



RNA interference technology in crop protection against arthropod pests, pathogens and nematodes

Moises Zotti,^{a*} Ericmar Avila dos Santos,^a Deise Cagliari,^a
Olivier Christiaens,^b Clauvis Nji Tizi Taning^b and Guy Smagghe^{b*} 



R & D pipeline

Dow AgroSciences with Monsanto

- ✓ SmartStax Pro, which incorporates RNAi technology to counter resistance to Bt (*Bacillus thuringiensis*) proteins used as insecticides, will be introduced in 2018-20.
- ✓ The main target is western corn rootworm (*Diabrotica virgifera virgifera*).
- ✓ Coleoptera, such as western corn rootworm and Colorado potato beetle (*Leptinotarsa decemlineata*), have proved to be very promising targets for control by RNAi and much commercial development of the technology has been focused on these pests.

Thank You