

Növényi szabályozó kis RNS-ek

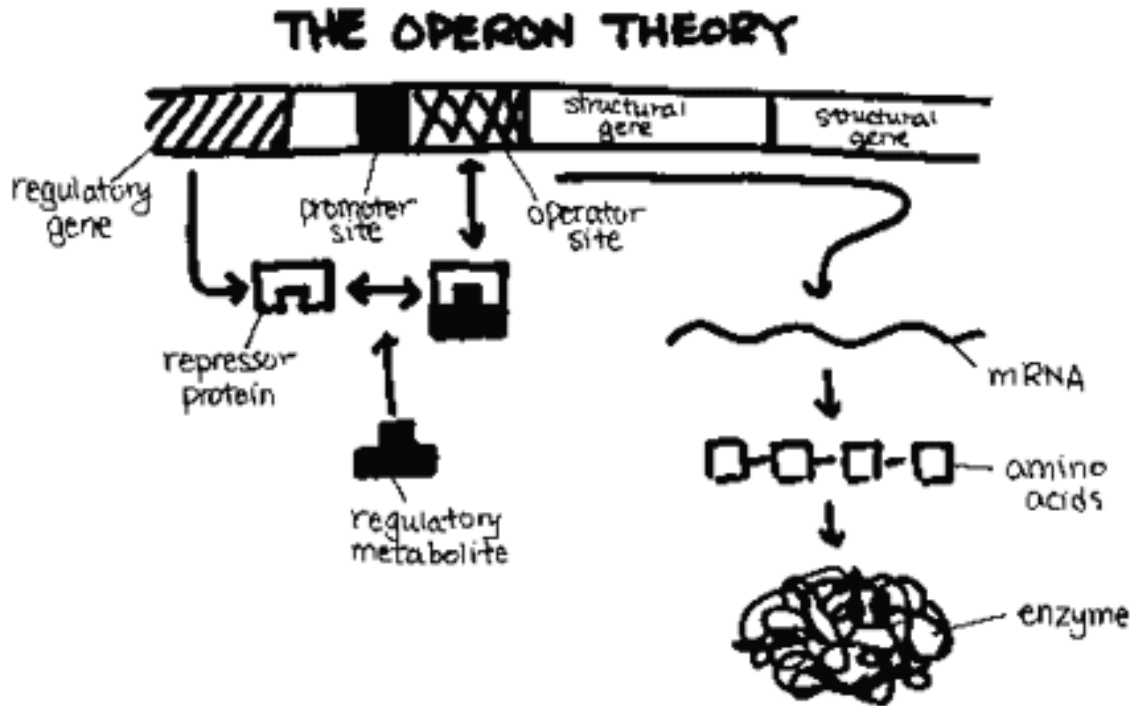
RNS csendesítés
RNA silencing
Poszt-transzkripcionális géncsendesítés
(PTGS)
RNS interferencia (RNAi)

Mezőgazdasági Biotechnológiai Kutatóközpont, Gödöllő
Havelda Zoltán, havelda@abc.hu

ELTE 2016



The Nobel Prize in Physiology or Medicine 1965 was awarded jointly to François Jacob, André Lwoff and Jacques Monod *"for their discoveries concerning genetic control of enzyme and virus synthesis"*.

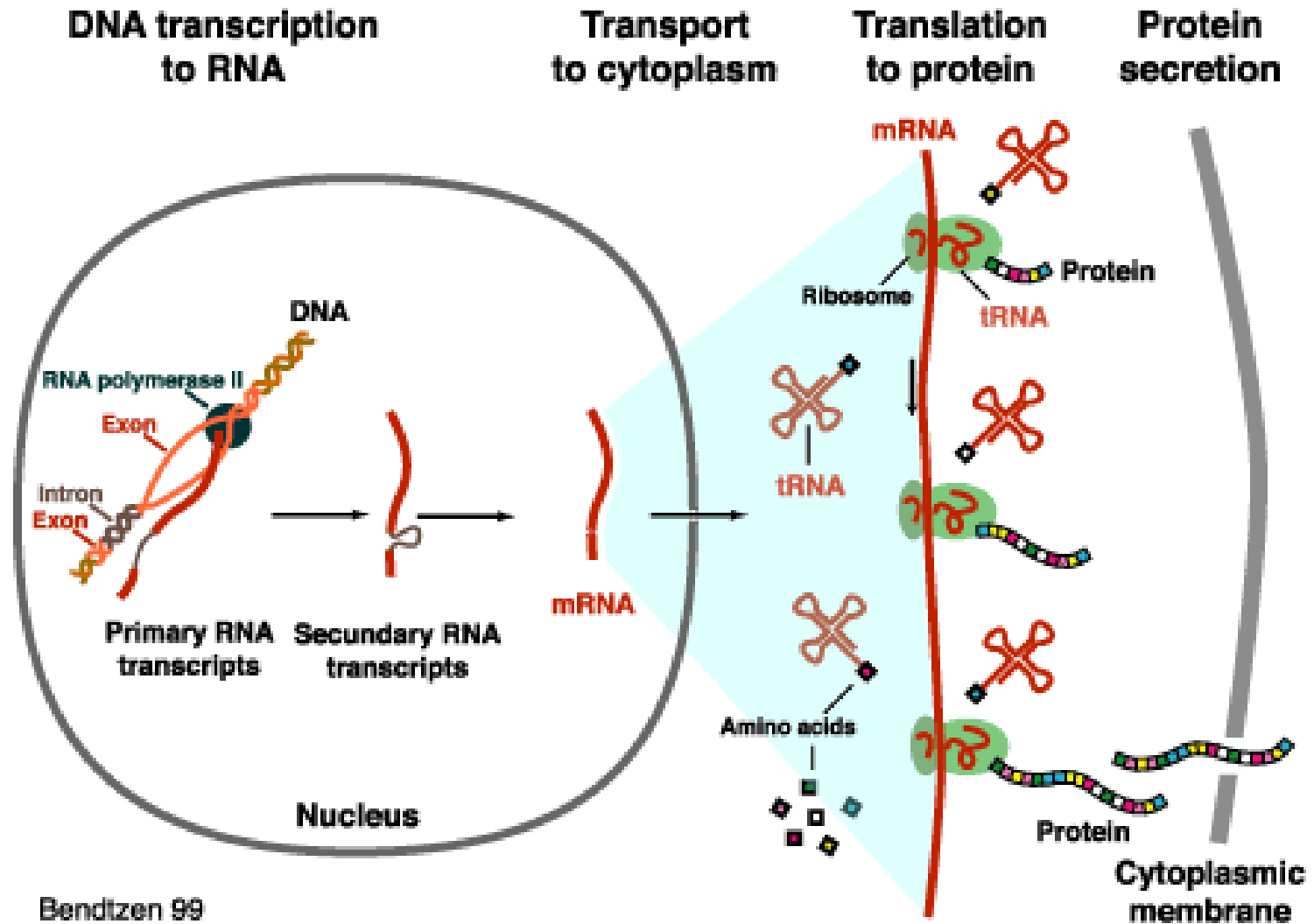


Jacob, Monod és Lwoff

Génexpresszió szabályozásának alapjai:

Az RNS-ek szerkezeti (rRNS), vagy transzfer (tRNS) vagy hirtívő (mRNS) szerepet játszanak

Mit is jellent ez?



Koszupresszió



Chalcone synthase gén pigment képzésért felelős

Erős promóter



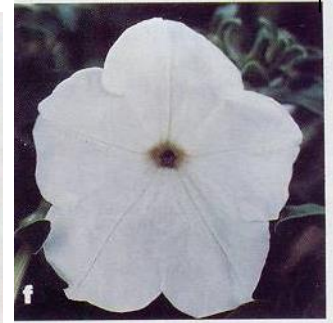
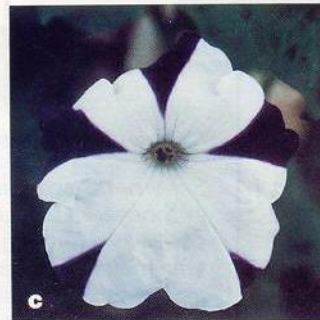
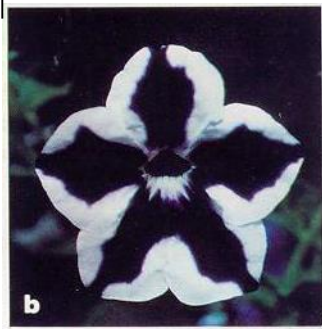
Terminátor



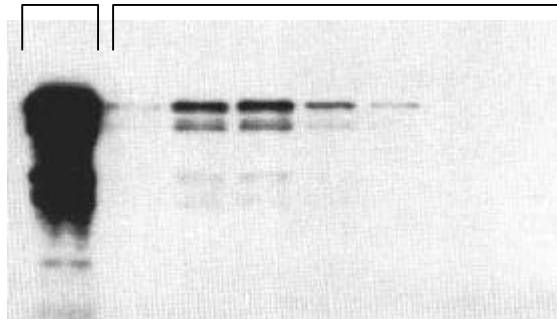
Chalcone synthase gén



Chalcone synthase transzgénikus petúnia vonalak



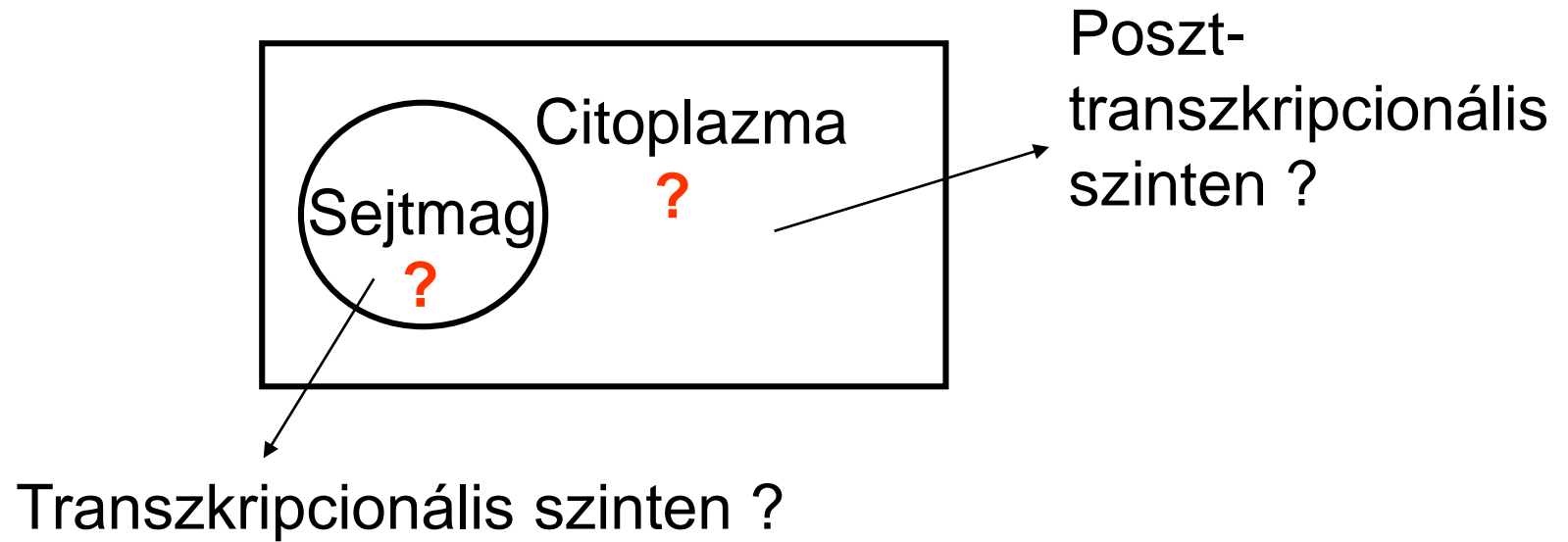
Nem transzformált transzformánsok



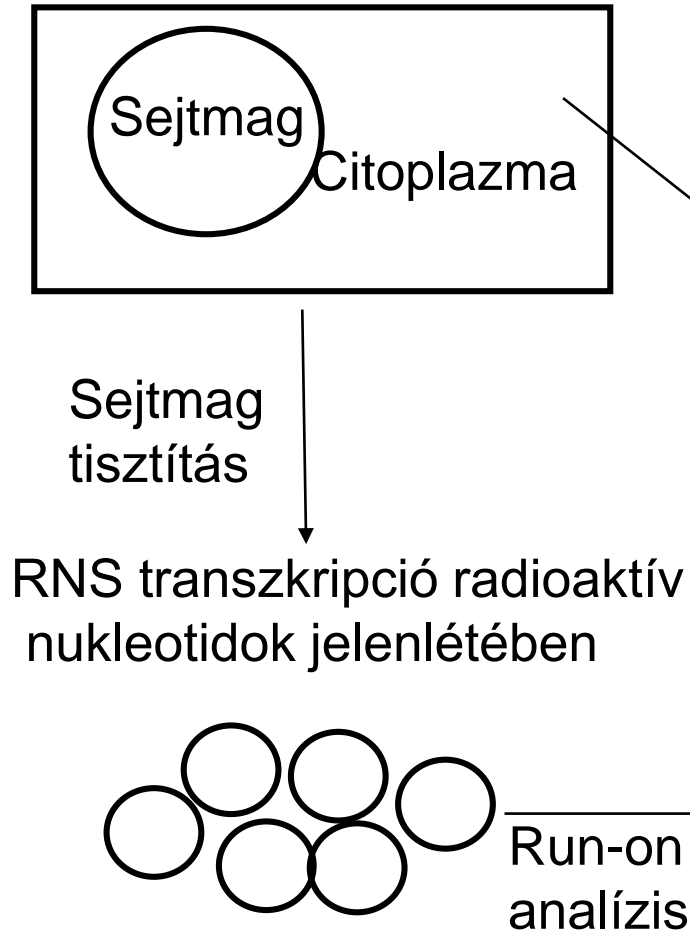
Chs mRNS Northern blot analízis

Napoli *et al.* 1990

Hol tűnik el a mRNS?



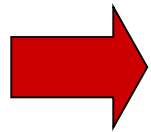
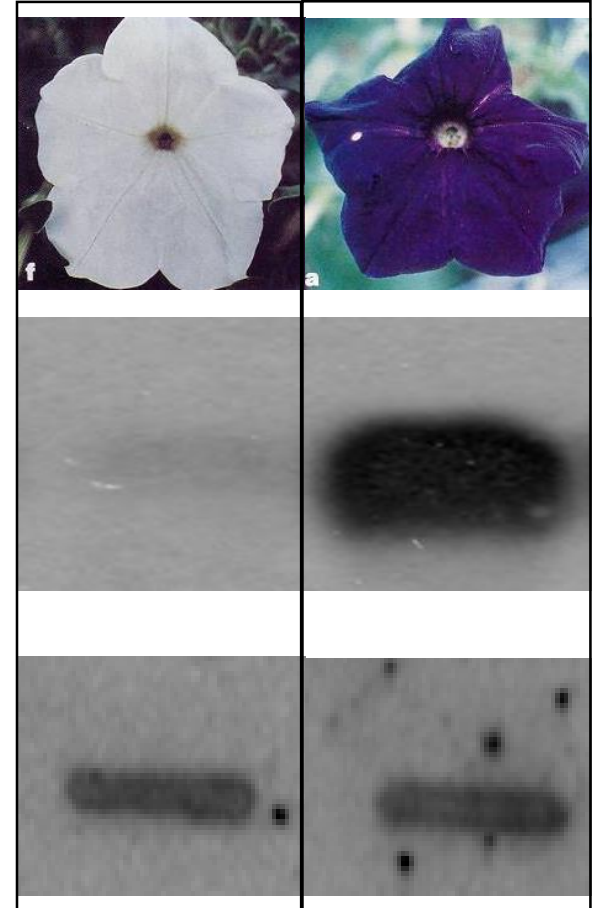
Run-on analízis



mRNS

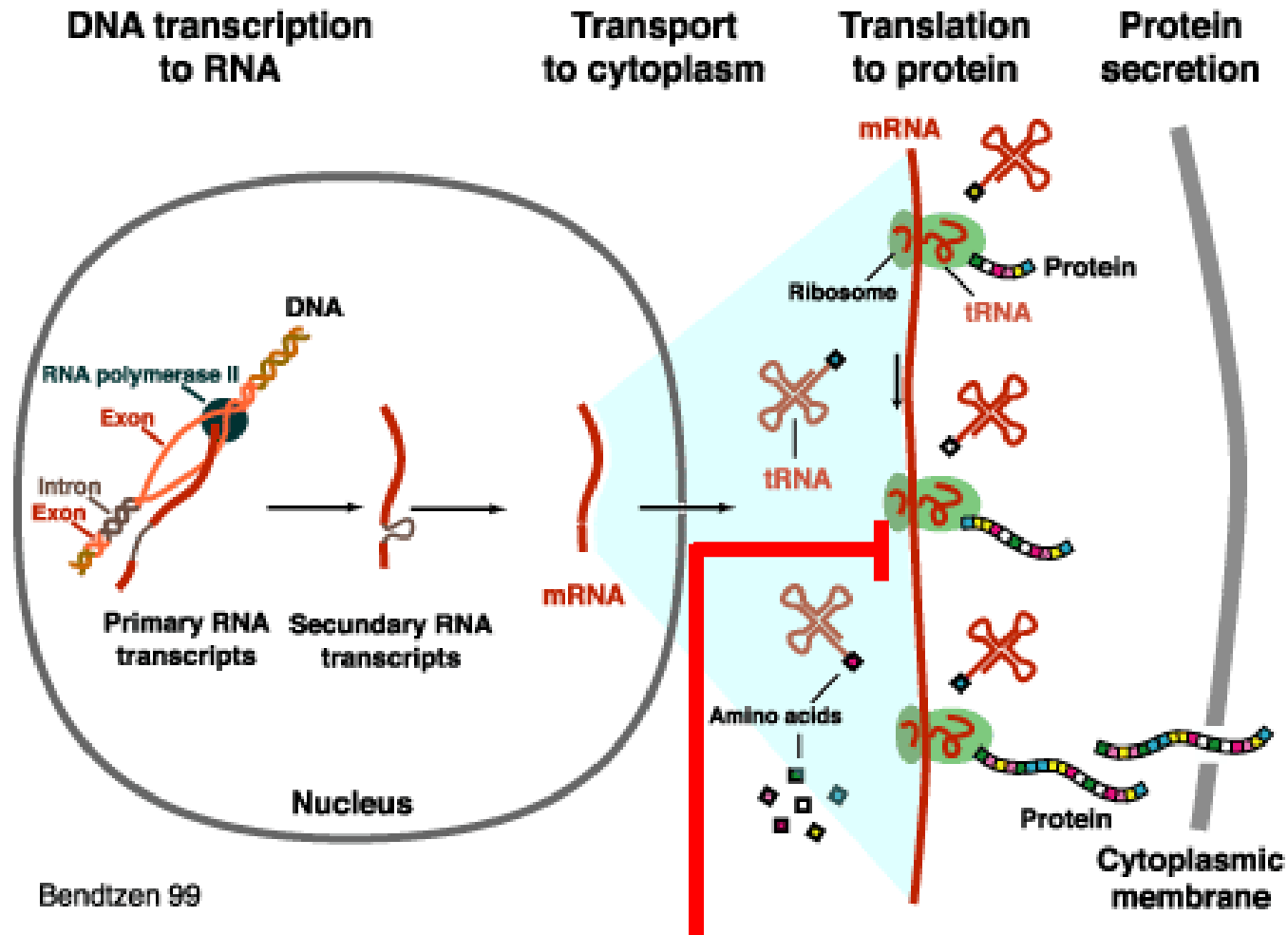
**A gén
sejtmagi
aktivitása**

Koszupresszált vonal
Vad típus



**Poszt-transzkripcionális RNS degradáció
(RNA silencing, RNA interference, PTGS)**

Egy új génszabályozási mechanizmus megismerése !



Kis RNS alapú szabályozás

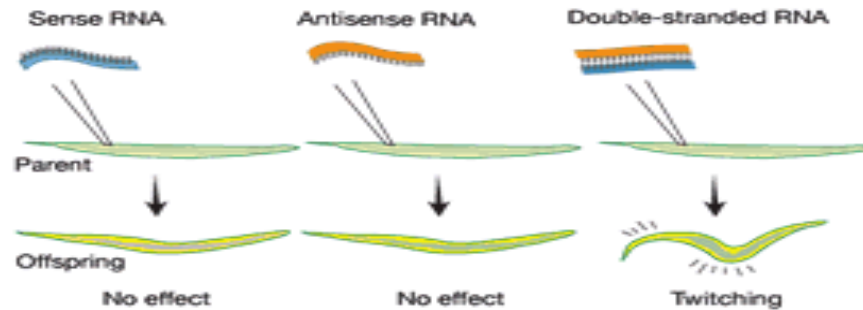
Mennyire általános az ilyen és ehhez hasonló interferencia alapú szabályozás az élővilágban?

Csak növényekre jellemző?

Állatokban is jelen van?

2. The experiment

RNA carrying the code for a muscle protein is injected into the worm *C. elegans*. Single-stranded RNA has no effect. But when double-stranded RNA is injected, the worm starts twitching in a similar way to worms carrying a defective gene for the muscle protein.

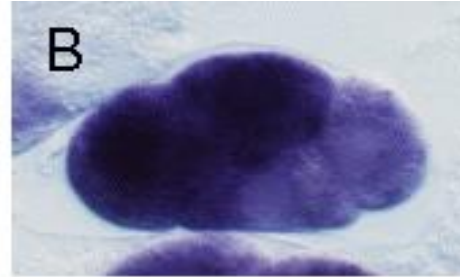


RNS interferencia *C. elegans*-ban

C. elegans embriók

Negatív kontroll

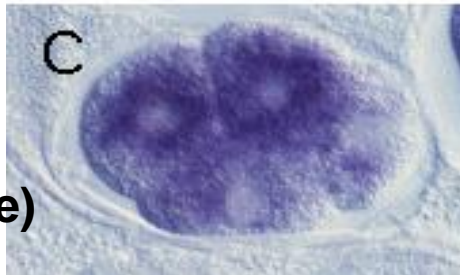
Nincs benne
mex-3 fehérje



Kezeletlen kontroll

Mex-3 fehérje
színreakciót ad

mex-3 egyszálú
RNS-sel kezelt
(sense vagy antisense)



mex-3 kétszálú
RNS-sel kezelt

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‡

* Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland 21210, USA

† Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA

‡ Program in Molecular Medicine, Department of Cell Biology, University of Massachusetts Cancer Center, Two Biotech Suite 213, 373 Plantation Street, Worcester, Massachusetts 01605, USA

Experimental introduction of RNA into cells can be used in certain biological systems to interfere with the function of an endogenous gene^{1,2}. Such effects have been proposed to result from a simple antisense mechanism that depends on hybridization between the injected RNA and endogenous messenger RNA transcripts. RNA interference has been used in the nematode *Caenorhabditis elegans* to manipulate gene expression^{3,4}. Here we investigate the requirements for structure and delivery of the interfering RNA. To our surprise, we found that double-stranded RNA was substantially more effective at producing interference than was either strand individually. After injection into adult animals, purified single strands had at most a modest effect, whereas double-stranded mixtures caused potent and specific interference. The effects of this interference were evident in both the injected animals and their progeny. Only a few molecules of injected double-stranded RNA were required per affected cell, arguing against stoichiometric interference with endogenous



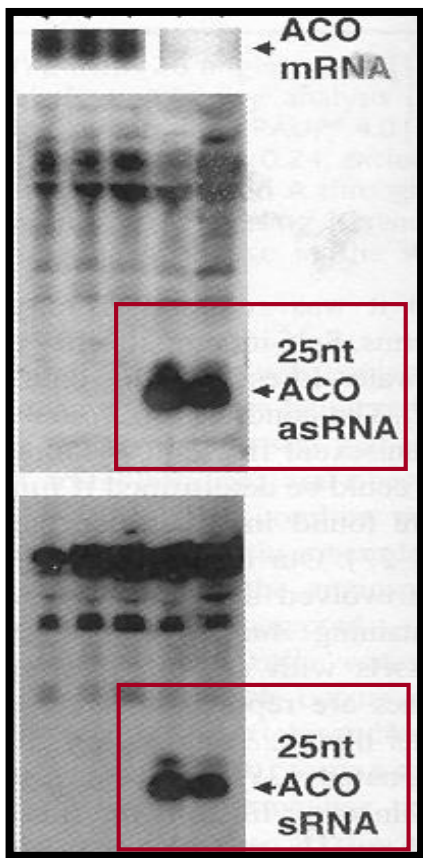
2006 Nobel Dĭj

NATURE | VOL 391 | 19
FEBRUARY 1998 807

Az RNS interferencia általános mechanizmusa

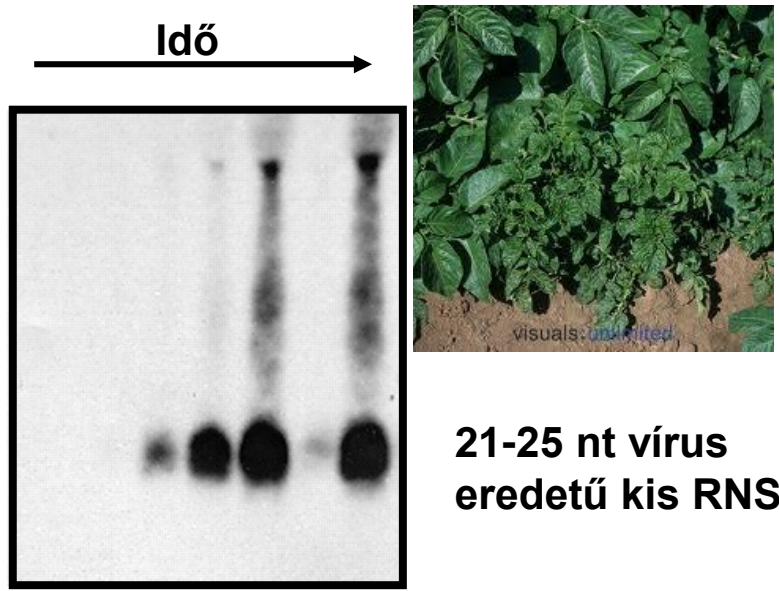
RNS interferenciával társult kis RNS-ek felfedezése növényekben

NEM mutat
RNS interferenciát
RNS interferenciát
mutat



ACO transzgénikus paradicsom vonalak

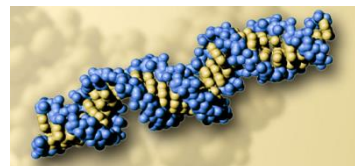
Burgonya X vírus fertőzött növény



anti-sense
próba

sense
próba

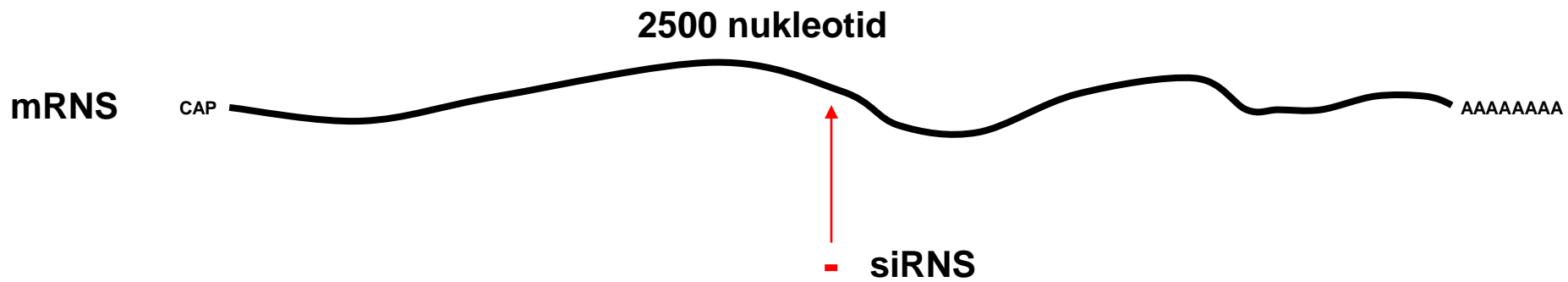
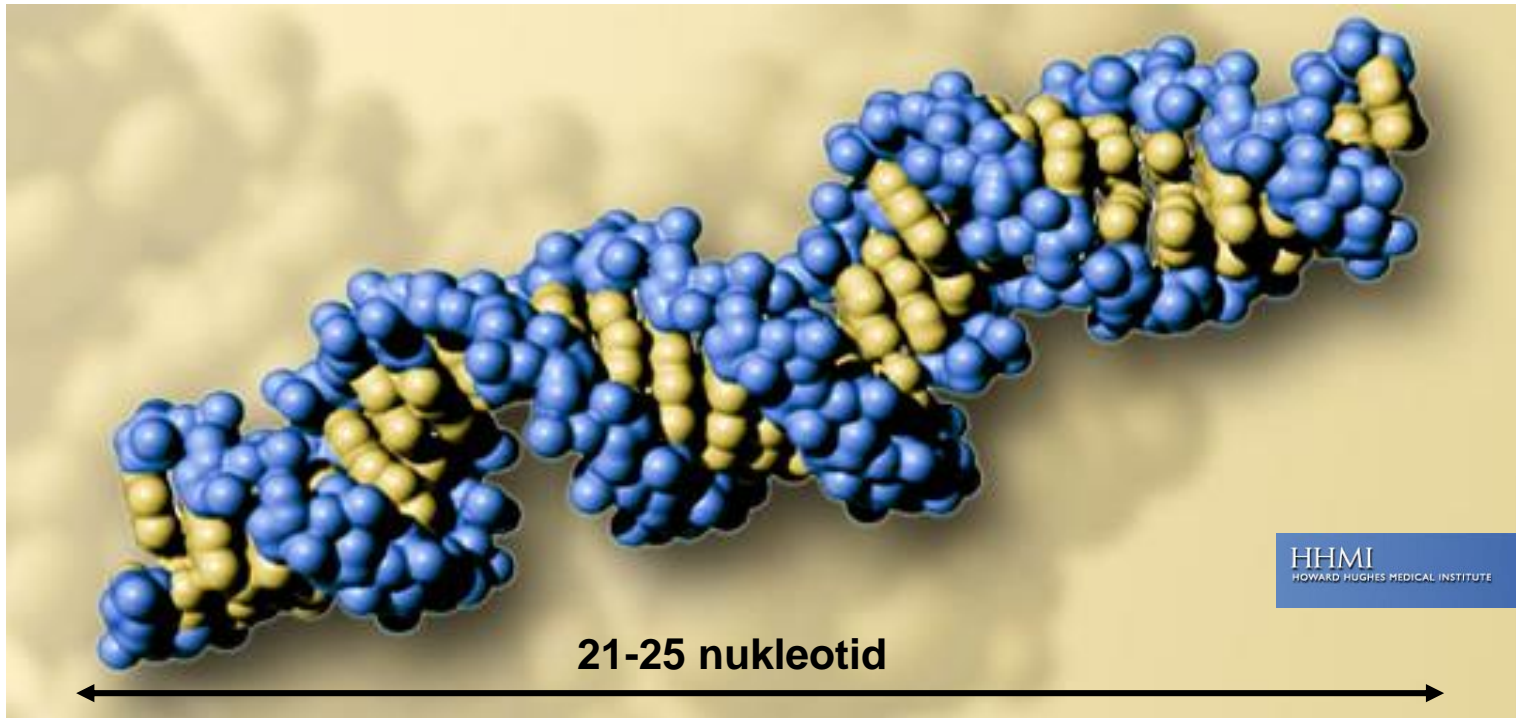
!



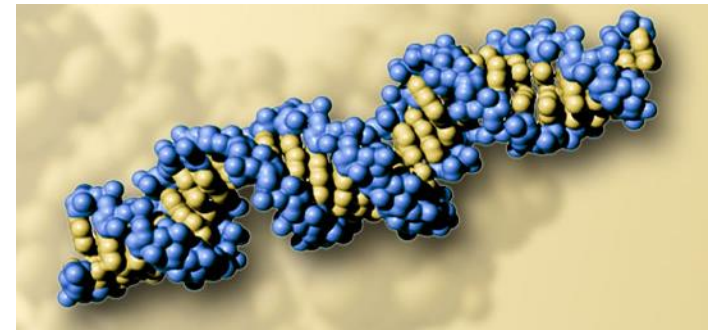
Kis interferáló RNS-ek (small interfering; siRNA)

siRNS

(small interfering RNA; kis interferáló RNS)



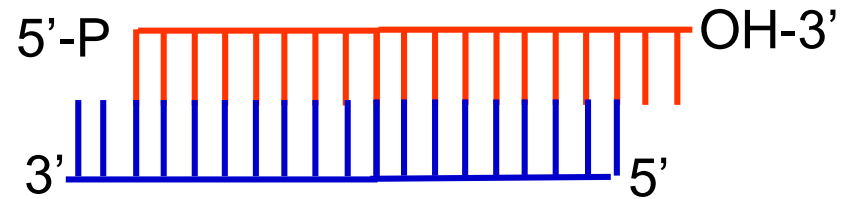
siRNS-ek jellemzői



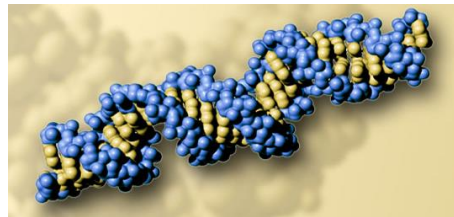
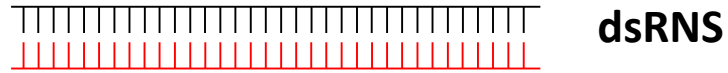
← 21-25 nt →

- 21-25 nt dsRNS-ek,
- 2 nt 3' túlnyúló véggel
- 5' foszforilált

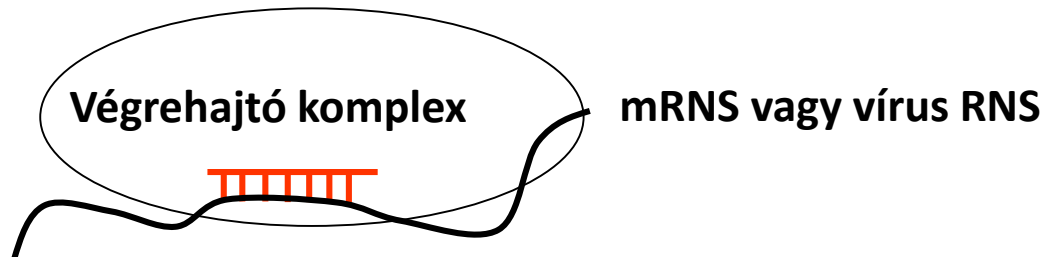
(-3' metilált (növények))



RNS alapú szabályozás



21-25 nt siRNS



miRNS-ek

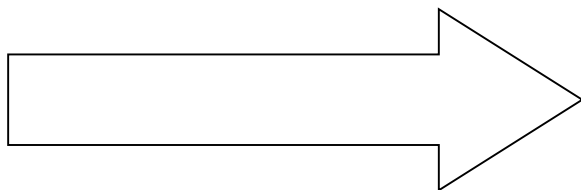
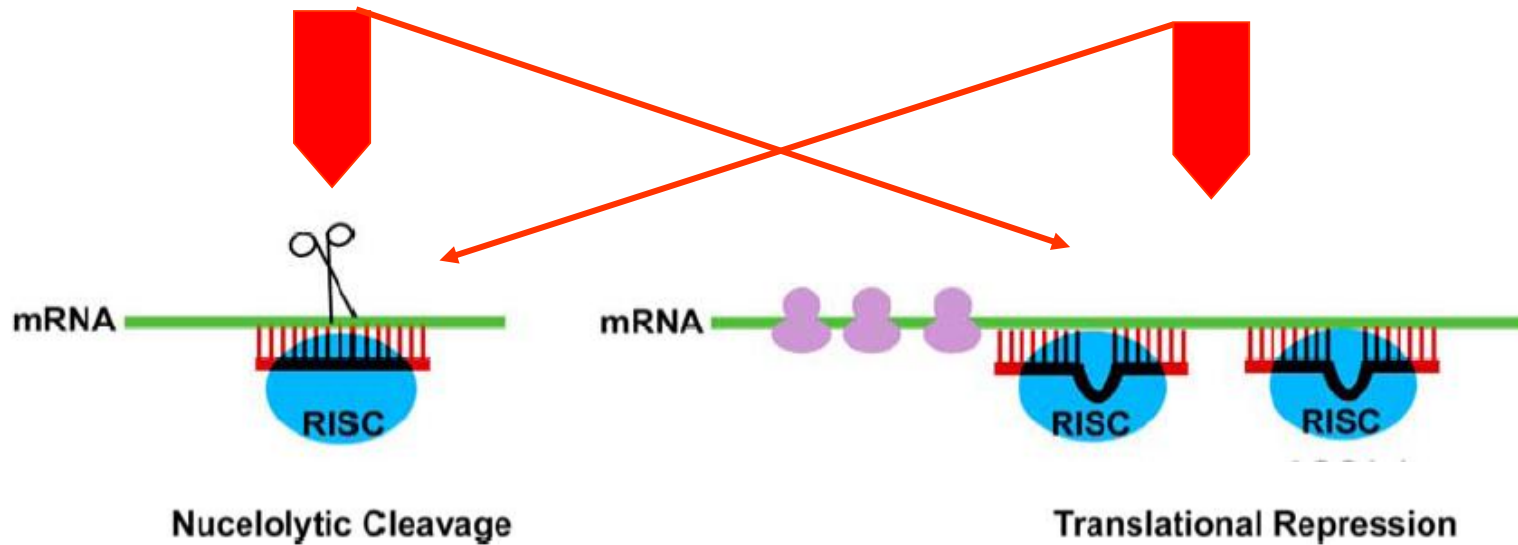
mRNS

szekvensspecifikus hasítása

a transzláció attenuációja,

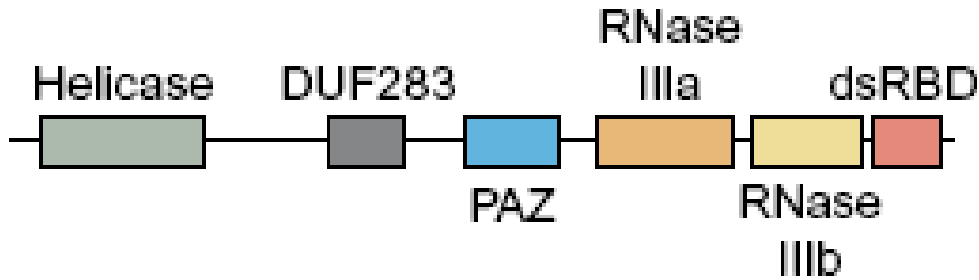
NÖVÉNYEK

ÁLLATOK



Fejlődési folyamatokat és endogén gének kifejeződését szabályozzák

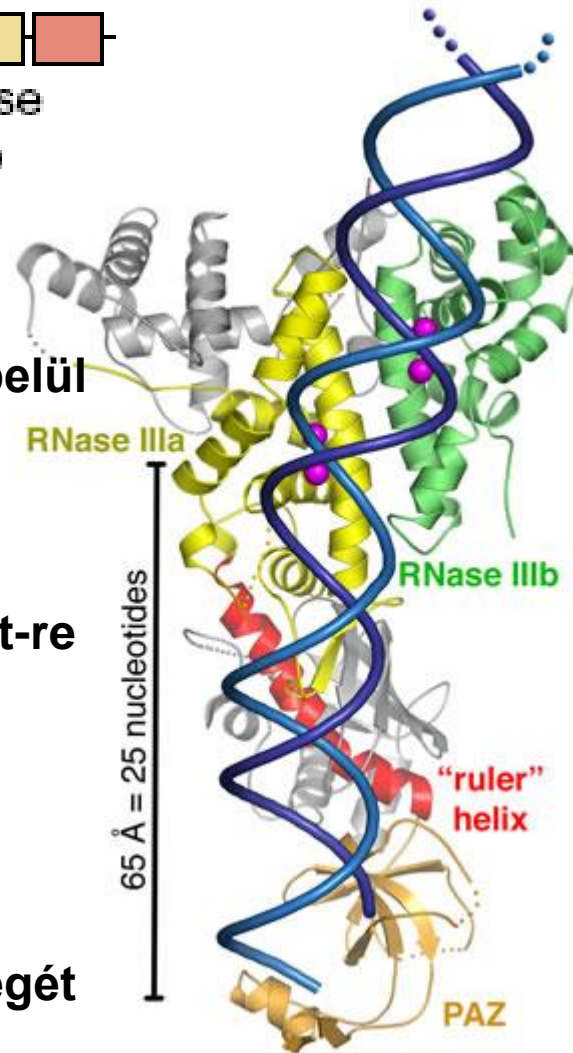
DICER



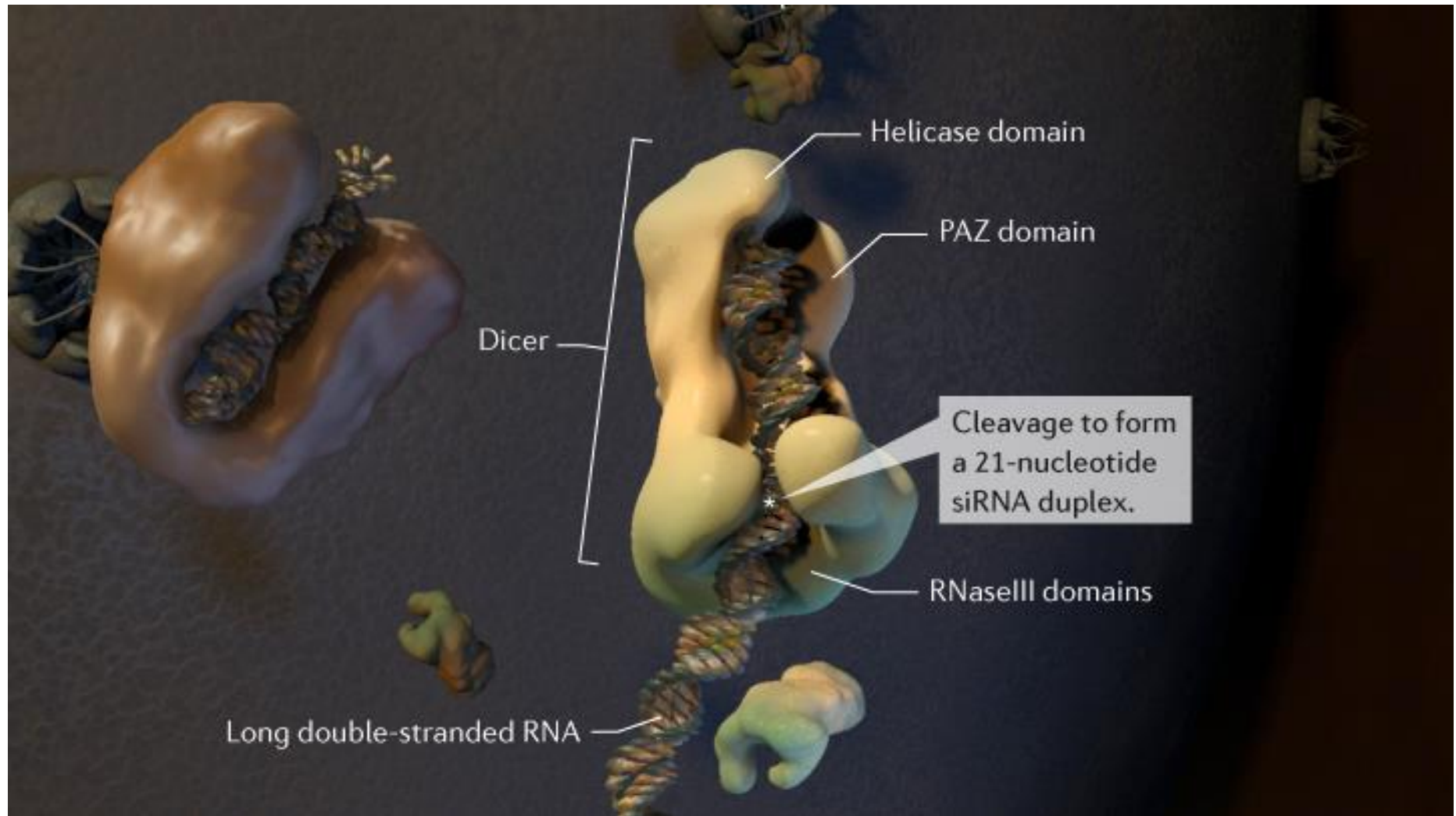
A két RN-áz III domén alakítja ki a végrehajtó központot, molekulán belül

A PAZ és az RN-áz III domén közti különbség adja meg, hogy hány nt-re a végtől hasít

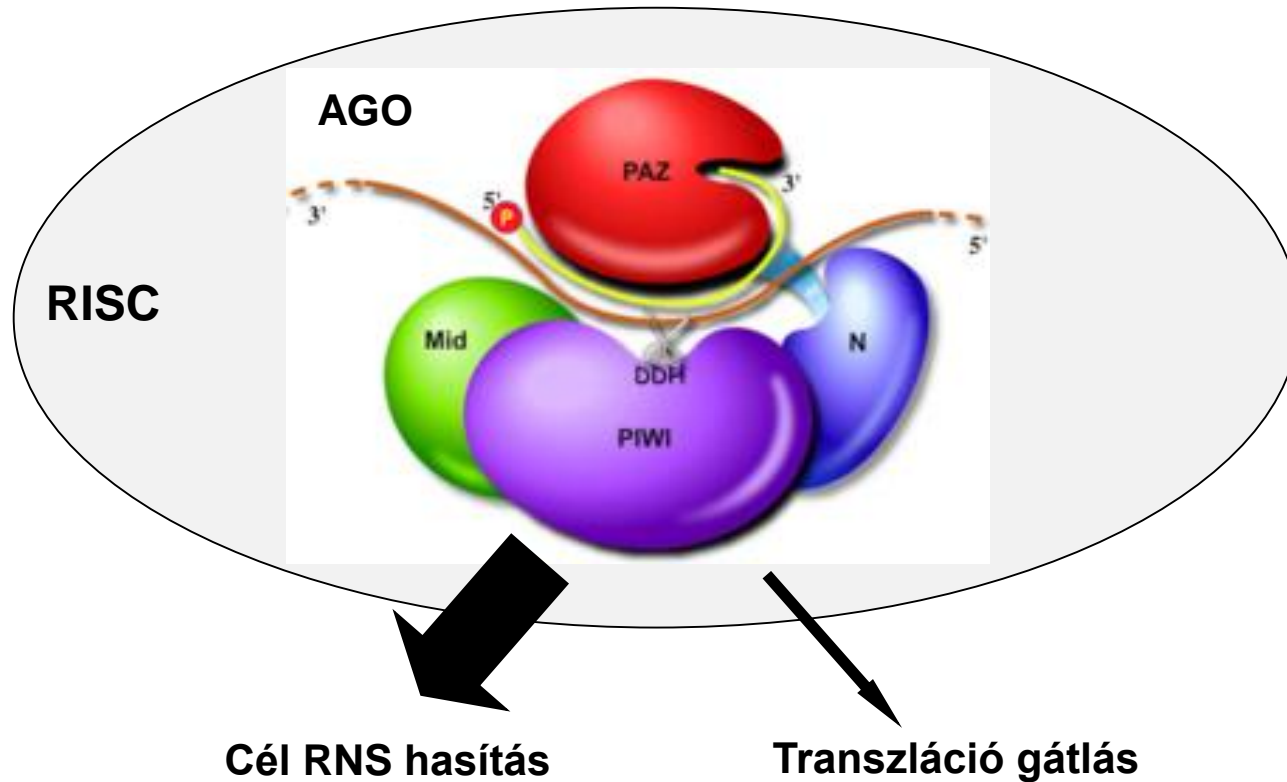
PAZ - Felismeri az RNS 3' végét



DICER



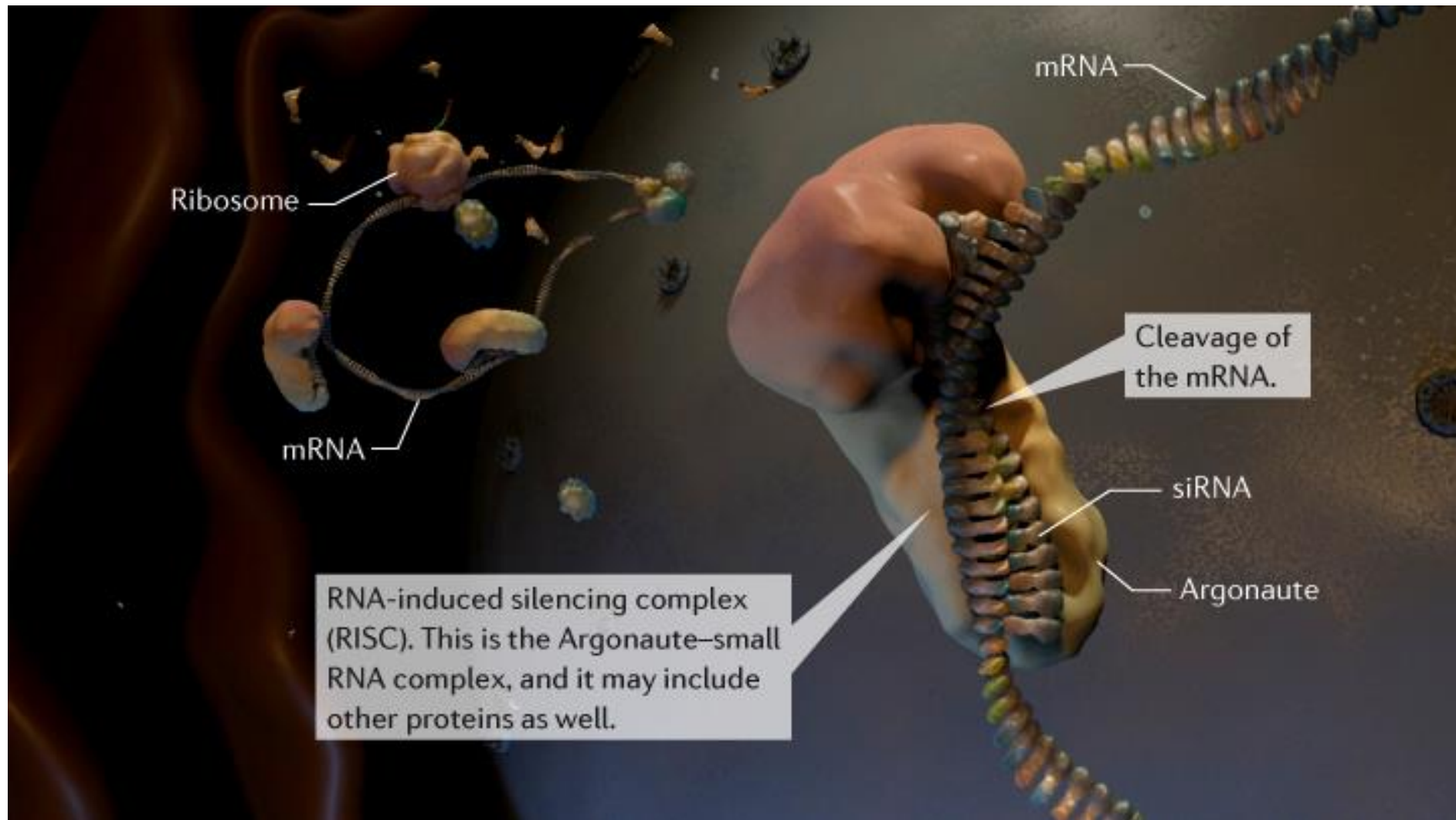
Az ARGONAUTE a RISC komplex központi molekulája



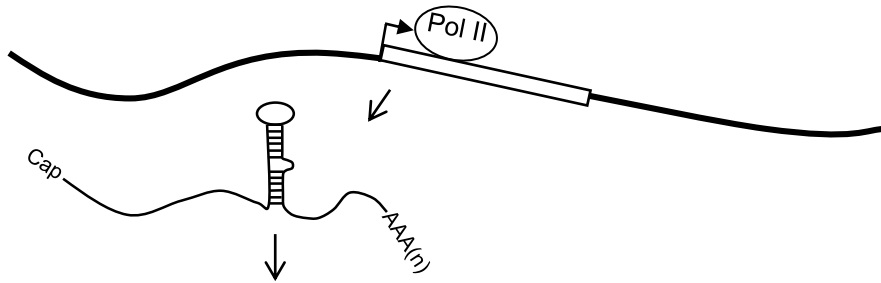
- A növényekben több Argonaute ortológ működik – Arabidopsisban 10
rizsben 18

- Az ortológok specializálódtak a különböző géncsendesítési útvonalakra

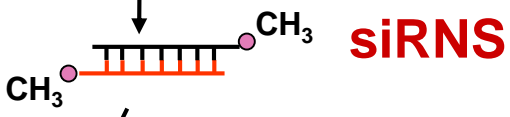
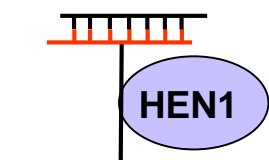
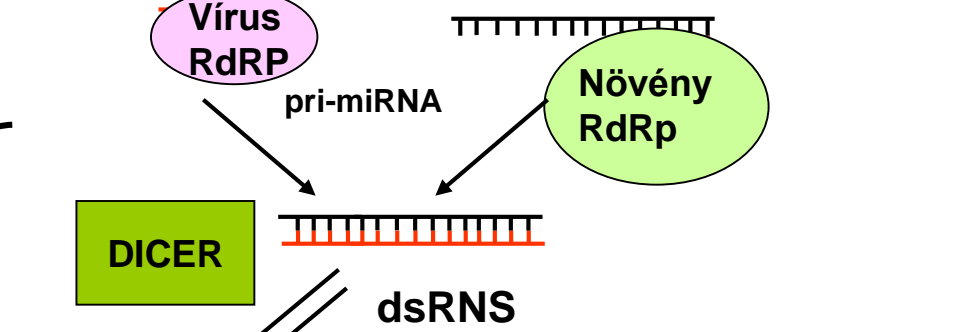
RISC – AGO fehérje



miRNS gének ! (Számuk nagyjából megegyezik a transzkripciós faktorok számával)

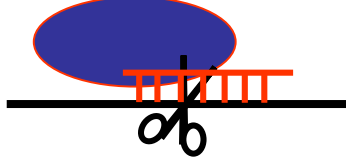


Vírus RNS miR locus Aberráns RNS (transzgén)

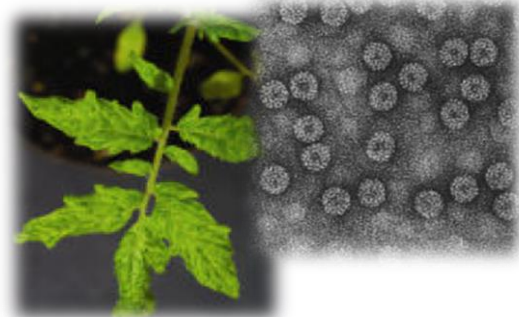


siRNS alapú RNS interferencia működése növényben

ArGOnaute (AGO)



Cél RNS hasítás / Transzláció gátlás



Szabályozó kis RNS-ek típusai

Osztály	Biogenezis	Funkció
Elsődleges siRNS-ek	kettős szálú RNS, hajtűszerkezet (DICER enzimek processzálják)	kapcsolódás a cél RNS-hez, másodlagos siRNS szintézis
Másodlagos siRNS-ek	RdRp (RNS függő RNS polimeráz) (DICER enzimek processzálják)	PTGS, heterokromatin kialakítása, fenntartása
Micro(mi)RNS-ek	miRNS prekursorok (miRNS gének) (DICER és RNaseIII szerű enzimek)	PTGS
Trans-acting(tasi)RNS-ek	miRNS hasítás indukálta dsRNS (DICER enzimek processzálják)	PTGS
Natural antisense transcript derived siRNS, natsiRNS	genomból származó dsRNS (DICER enzimek processzálják)	PTGS, növény (Patogenezis és stressz)
Piwi-interacting (pi)RNS	DICER független, de AGO függő	Transzpozonok, retroelemek szuppressziója (rovar, emlős)

Fejlődés biológiai folyamatok

Patogén elleni válaszok

Genom stabilitás fenntartása

**Micro(mi)RNS-ek
Fejlődés biológiai folyamatok
szabályozása**

miRNS-ek vannak növényekben is

Aktivációs tagging



jaw fenotípus



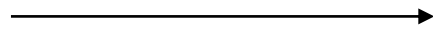
Microarray kísérlet

TCP géncsalád (levél fejlődés) tagjainak mRNS szintje jelentősen csökkent a mutánsban



Jaw locus (miRNS prekursor !!)

miR319



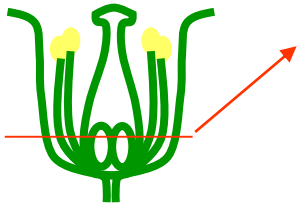
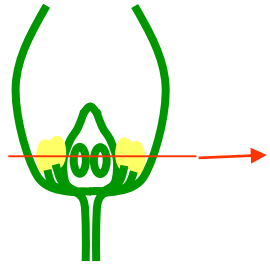
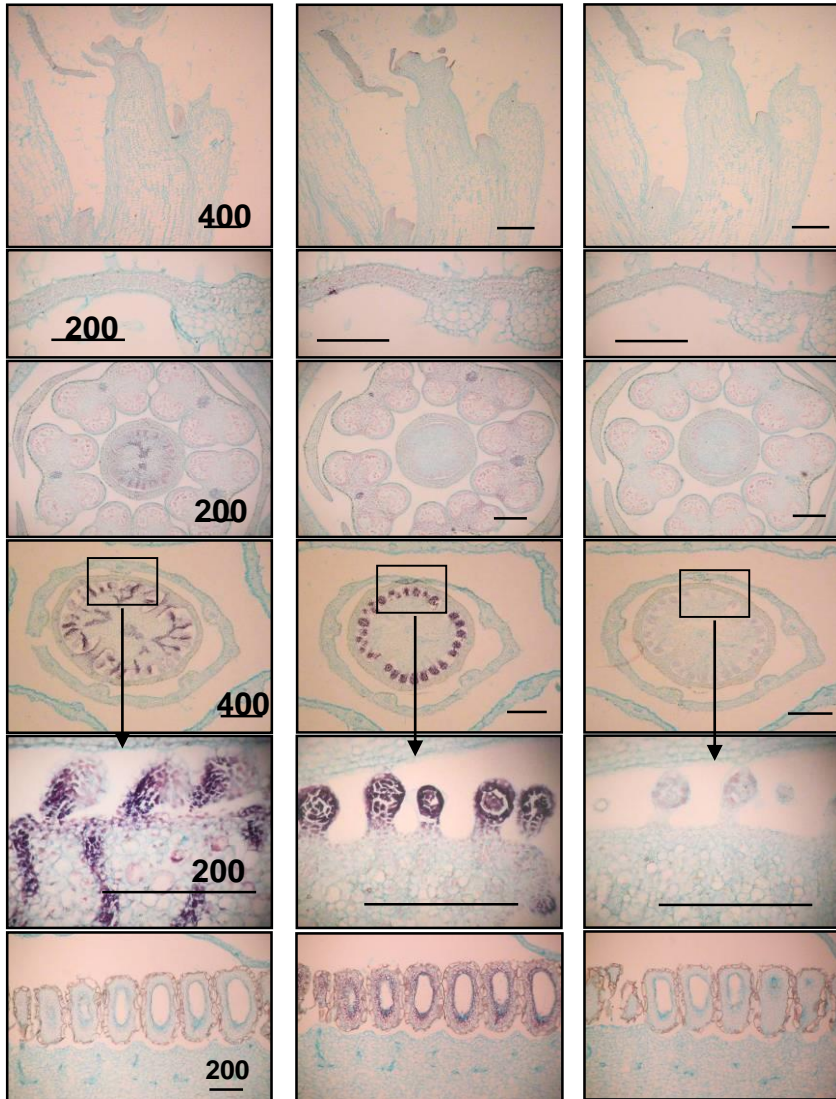
TCP gének degradációja

jaw növények transzformálva jaw miRNS rezisztens TCP génnel a növények normális fenotípust mutattak

Spatial accumulation of miR160 and miR167

Nicotiana benthamiana

miR160_LNA miR167_LNA - control

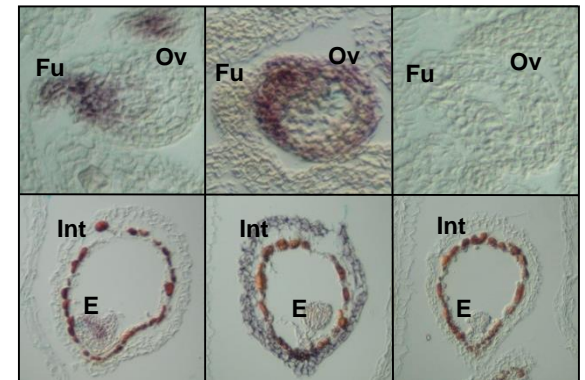


target:

ARF10, 16, 17 ARF6, 8
(Auxin response factor)

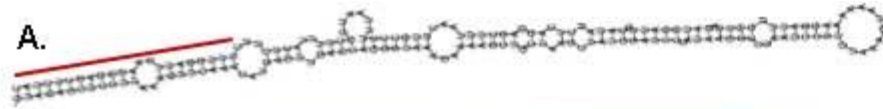
Arabidopsis thaliana

miR160 miR167 miR447

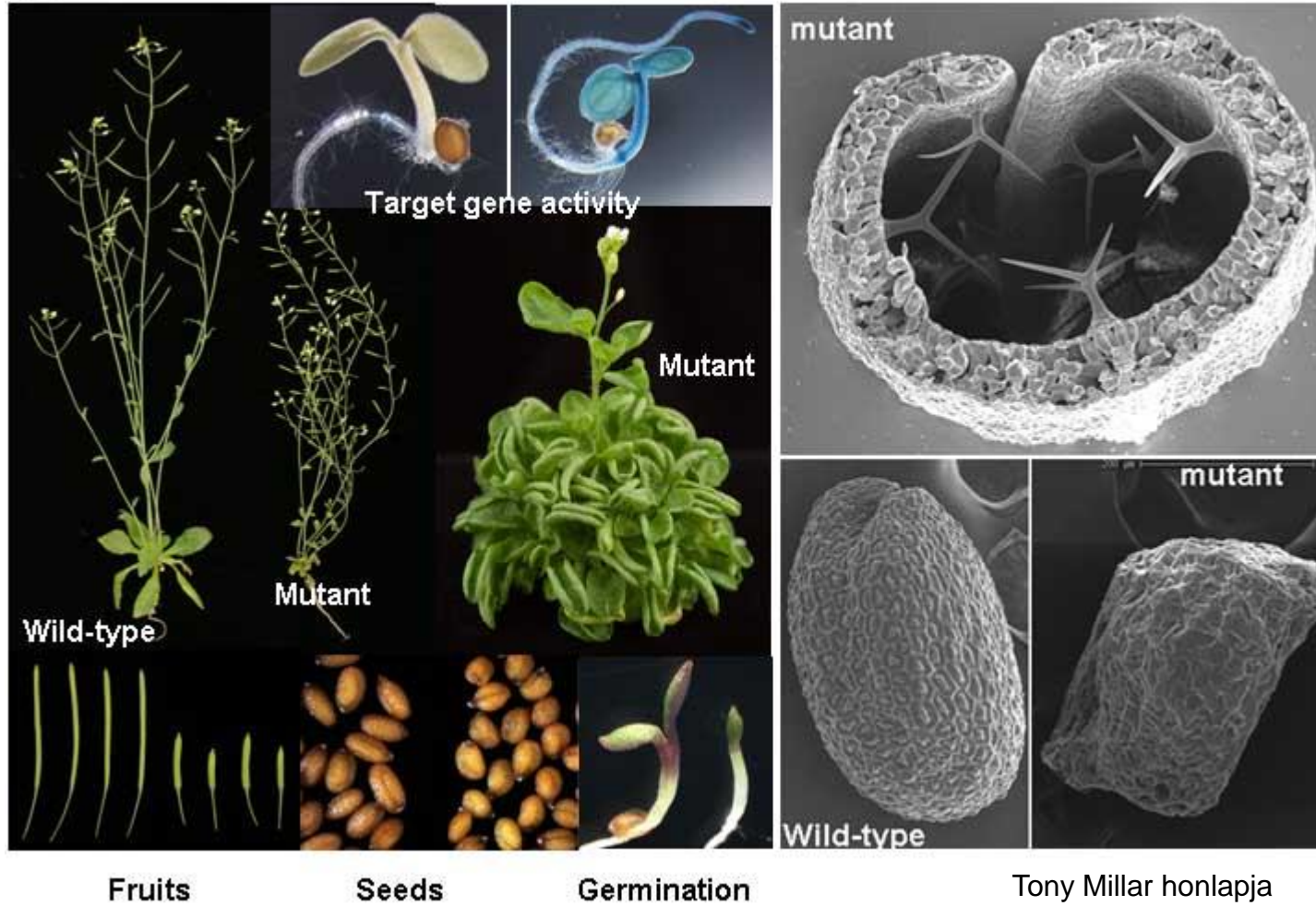


miR159 hiánya

A.



B. AUCUCGAGGGAAGUUAGGUUU





<http://microrna.sanger.ac.uk/sequences/index.shtml>

[Homo sapiens](#) (1881 precursors, 2588 mature)

[Mus musculus](#) (1193 precursors, 1915 mature)

[Arabidopsis thaliana](#) (325 precursors, 427 mature)

A genom 2% kódolhat miRNS-eket

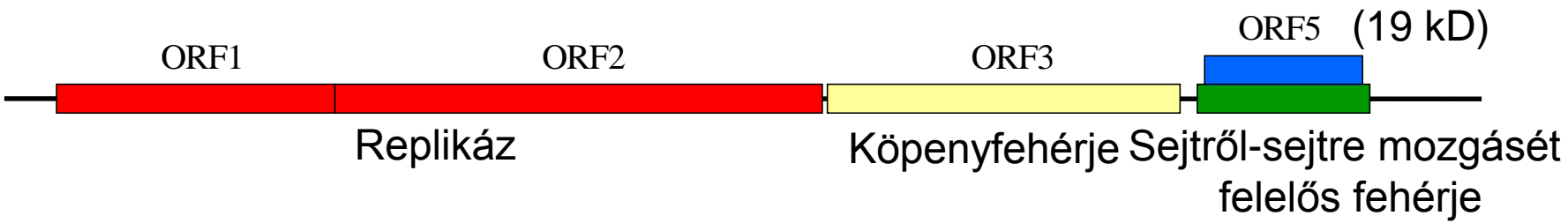
A géneknek 30% állhat az RNS csendesítés alapú kontroll alatt

Fejlődés biológiai folyamatok

Patogén elleni válaszok

Genom stabilitás fenntartása

Cymbidium gyűrűsfoltosság tombusvírus (CymRSV)



Kontroll



CymRSV fertőzött

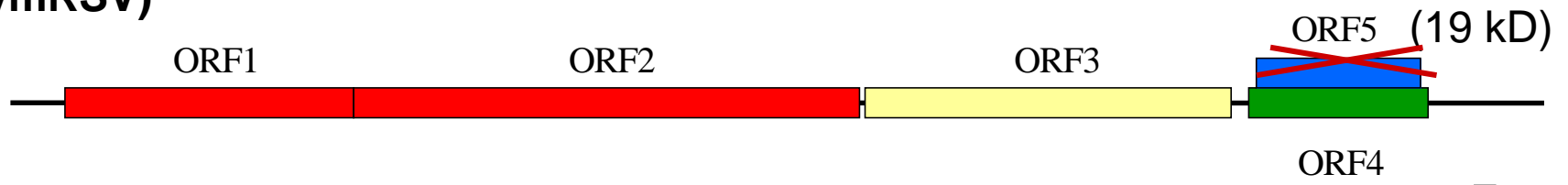


ORF4

Nicotiana benthamiana

P19 szerepe a vírus fertőzésben

(CymRSV)



Nicotiana benthamiana

Kontroll

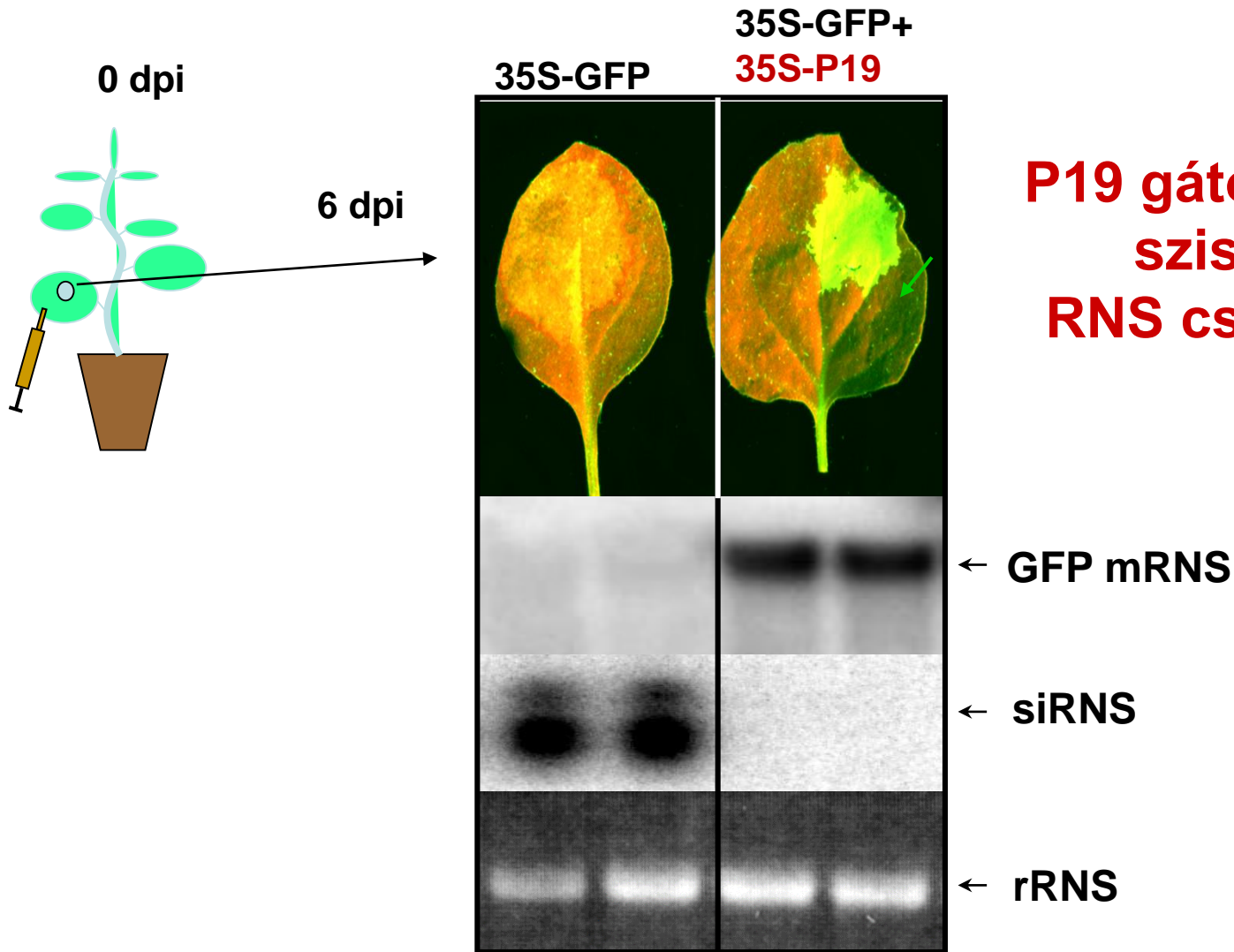
CymRSV

Cym19Stop



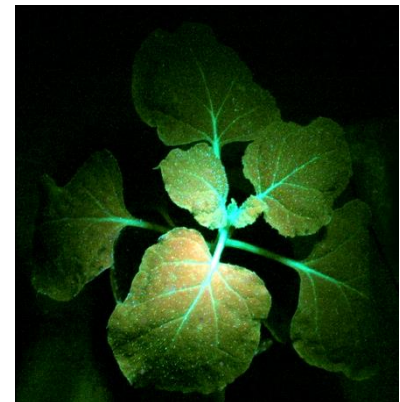
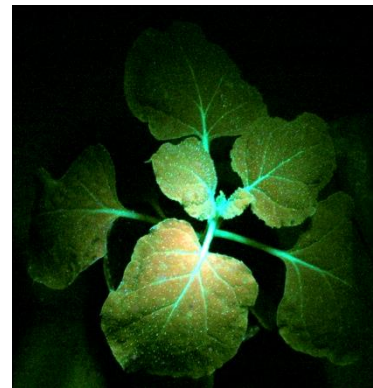
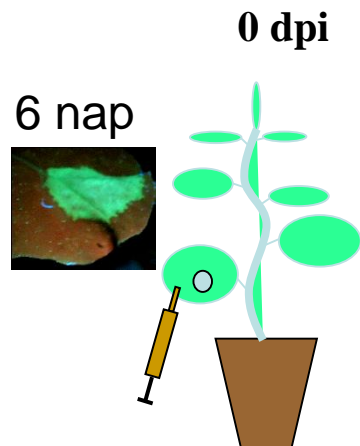
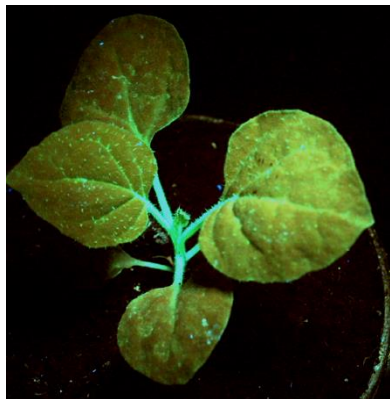
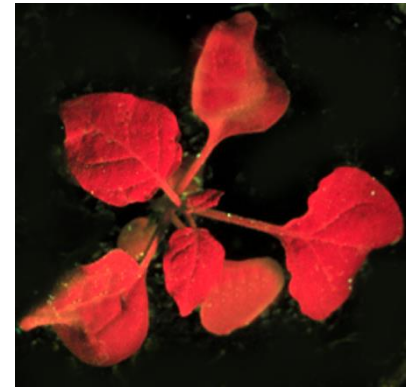
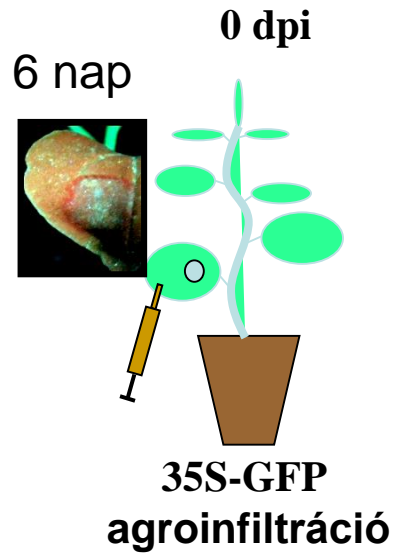
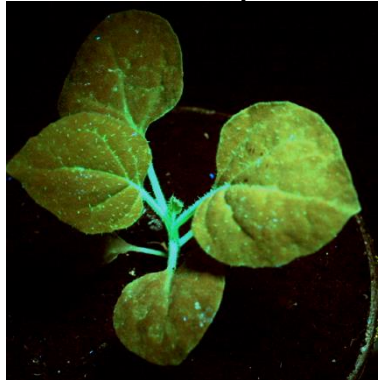
Kigyógyulás

P19 gátolja az transzgén indukált RNS csendesítés

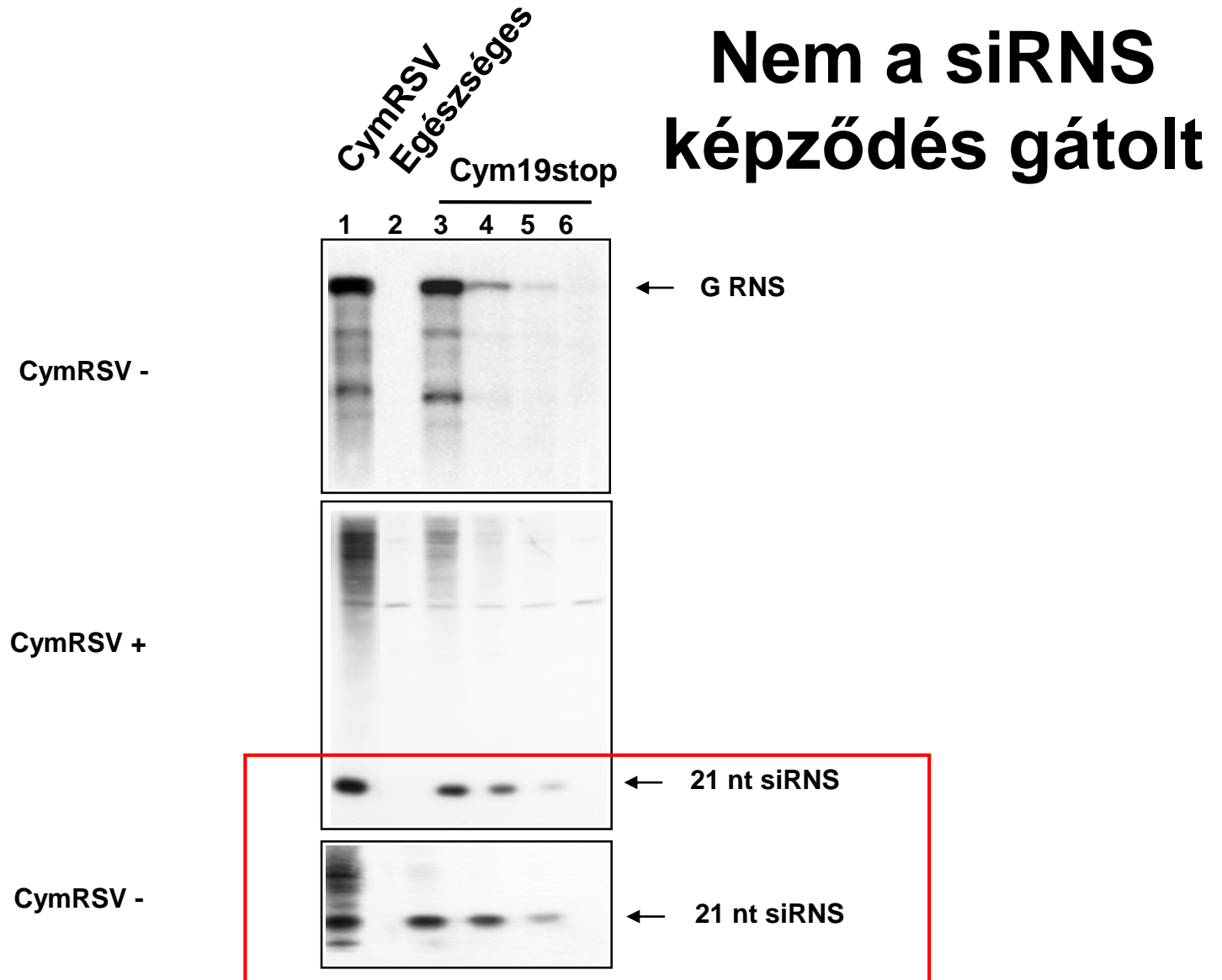


P19 gátolja a helyi és szisztémikus RNS csendesítést is

GFP expresszáló
növény



Vírus felhalmozódás P19 hiányában

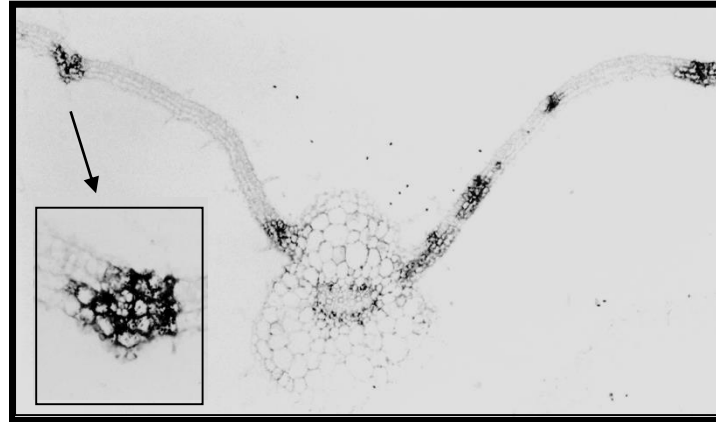


P19 hiányában a vírus terjedése lokalizálódik a növényben

CymRSV



Cym19Stop



Kontroll



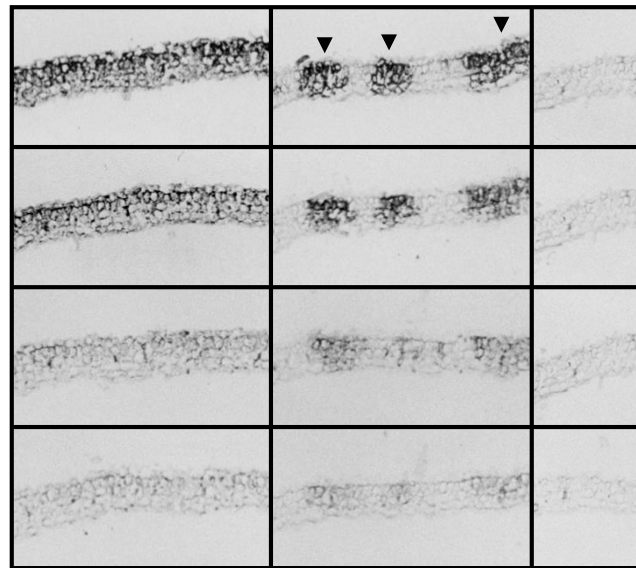
Hívási idő



CymRSV

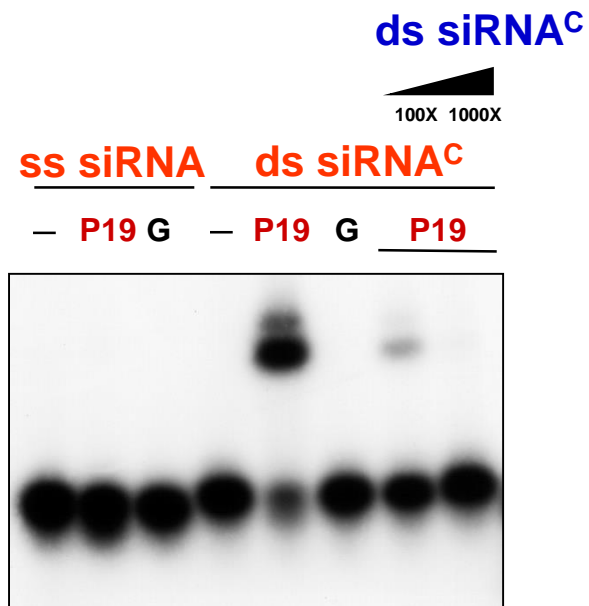
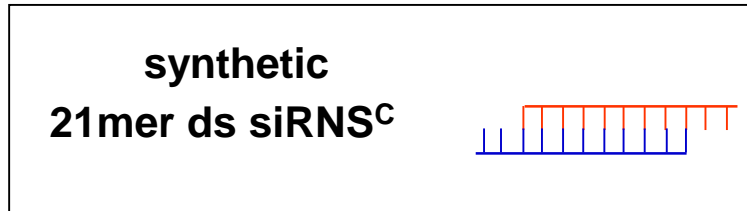
Cym19Stop

Kontroll

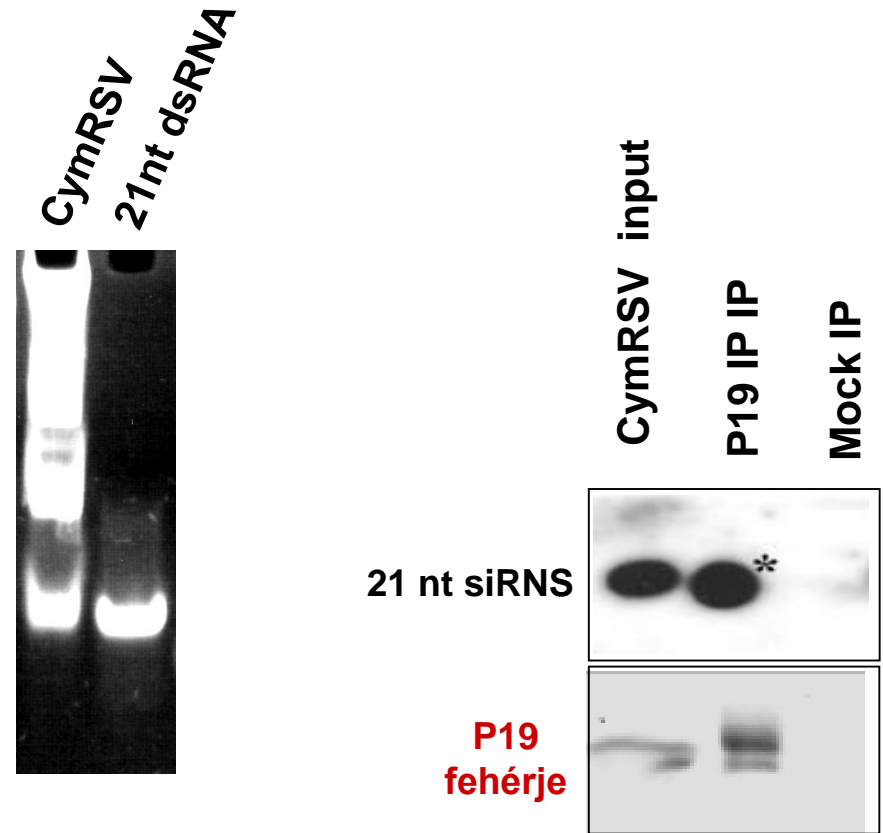


P19 specifikusan köti a siRNS-eket

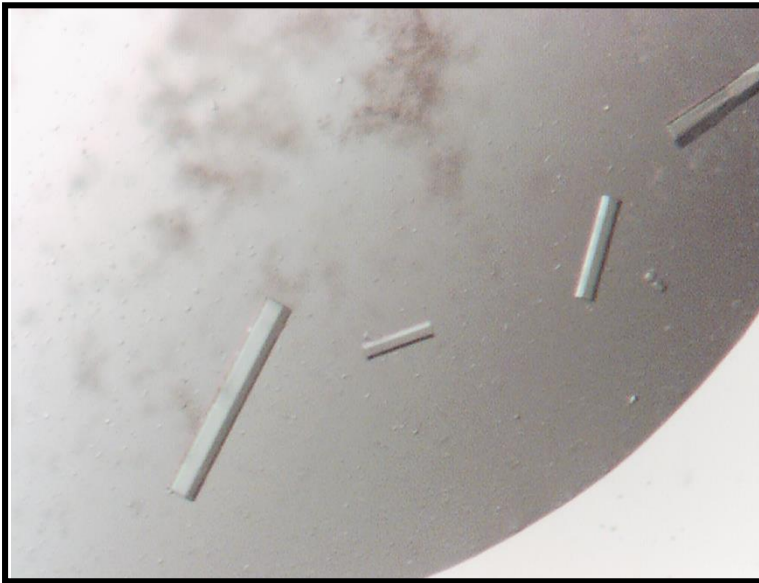
in vitro siRNS kötés vizsgálata



in vivo siRNS kötés vizsgálata

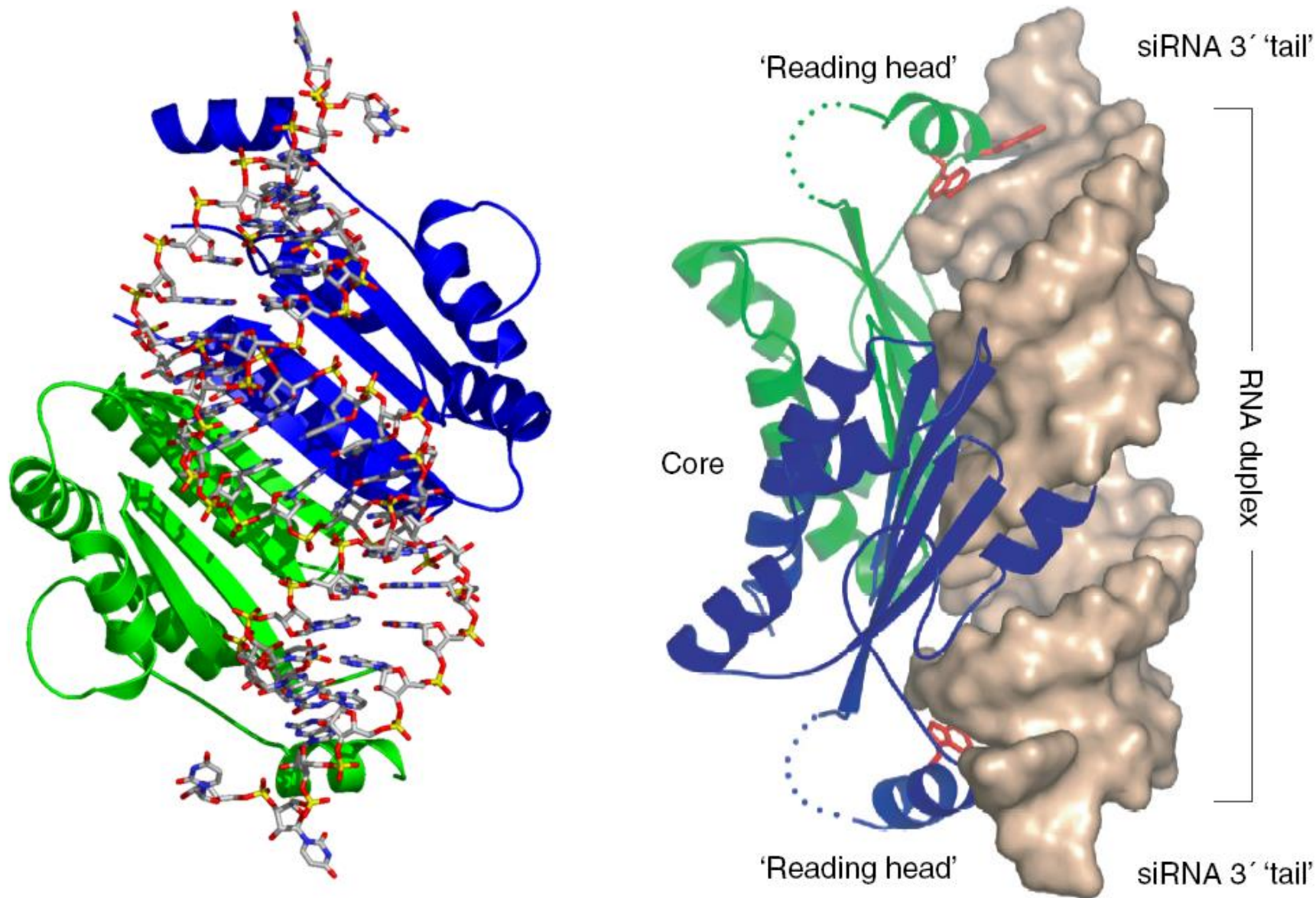


P19 fehérje szerkezete

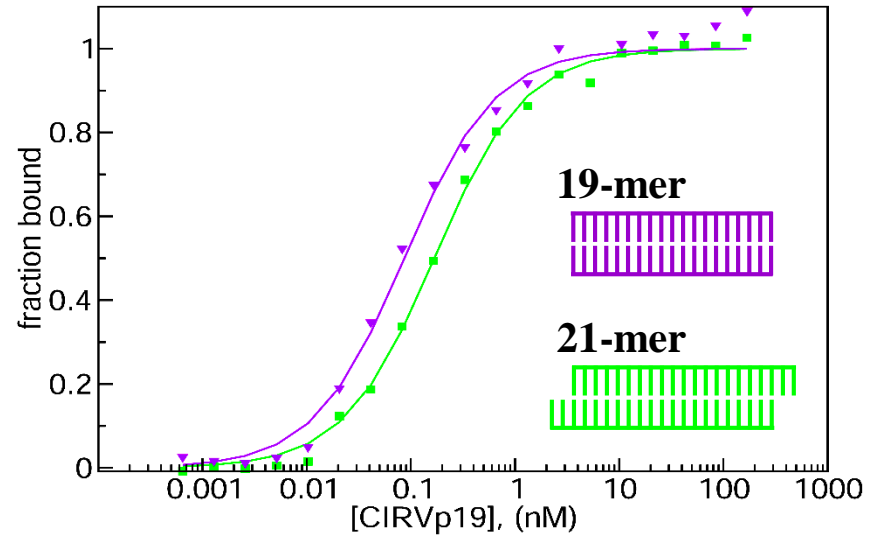
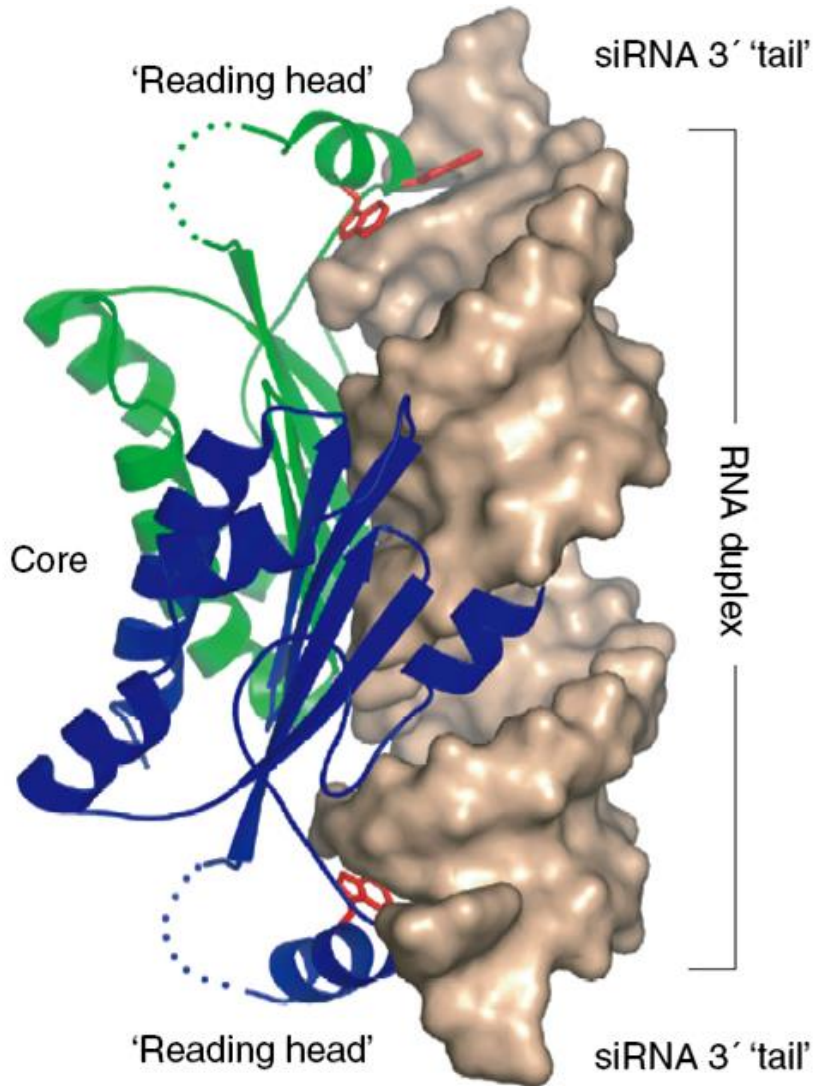


P19 ko-kristályok
21 nt siRNS-vel
komplexben

2.4 Å P19-ds-siRNS Komplex kristály szerkezete



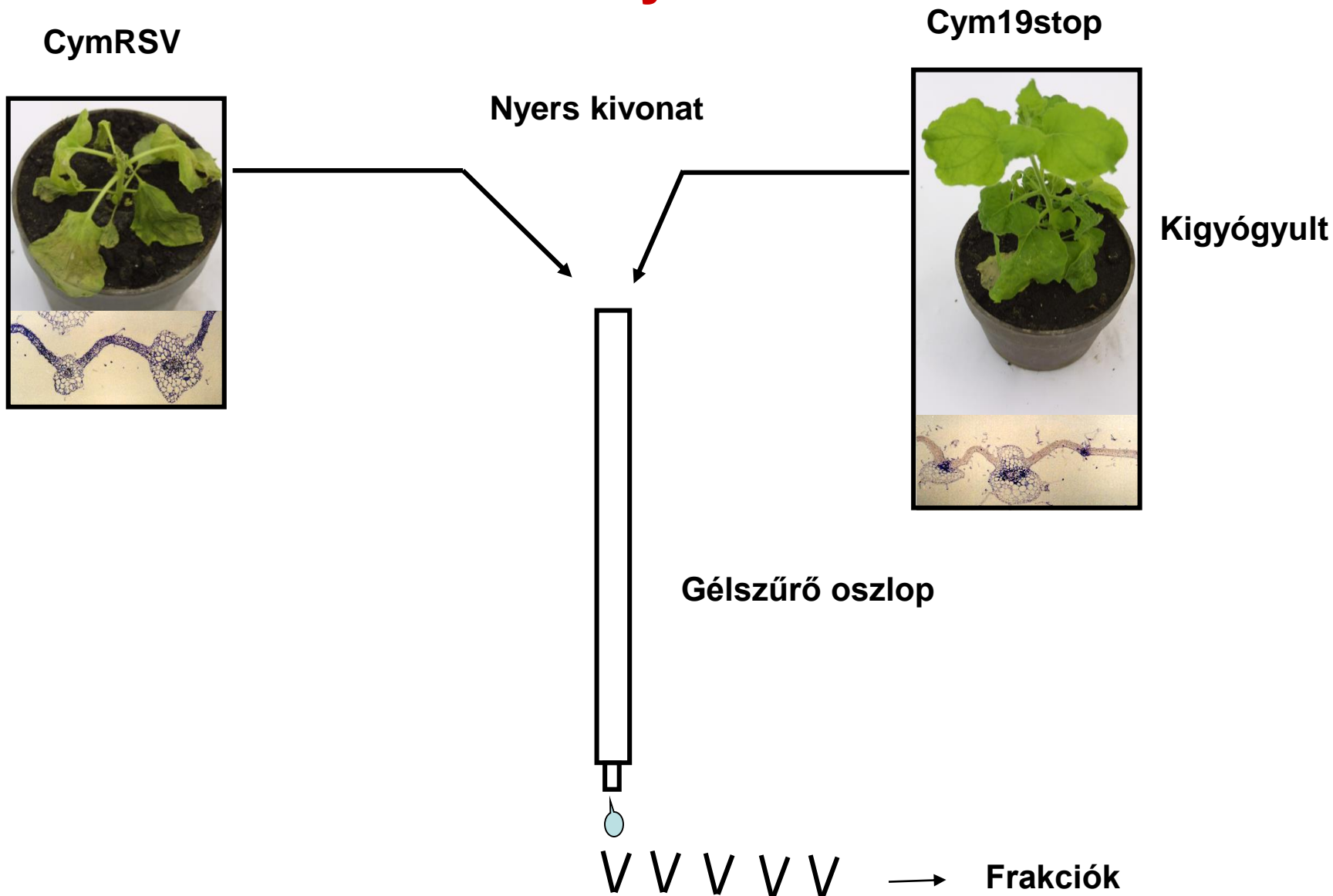
A siRNS 2 nt 3' túlnyúló vége nem szükséges az P19 kötéséhez

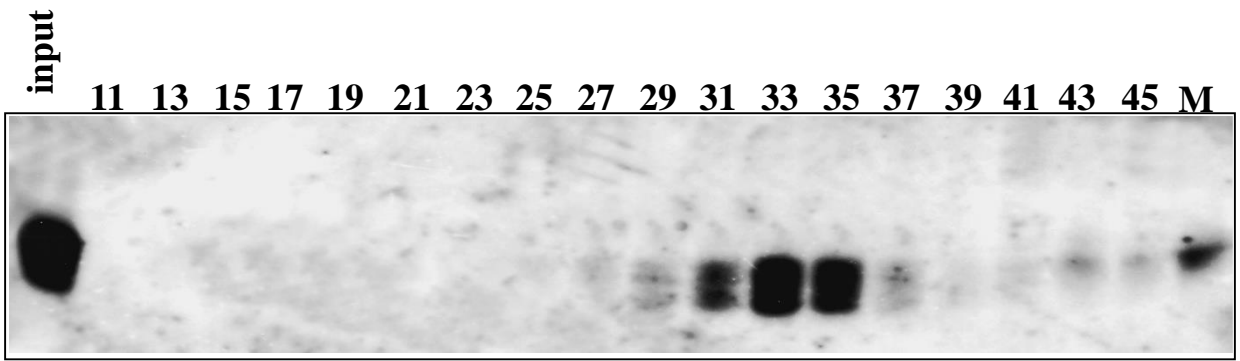
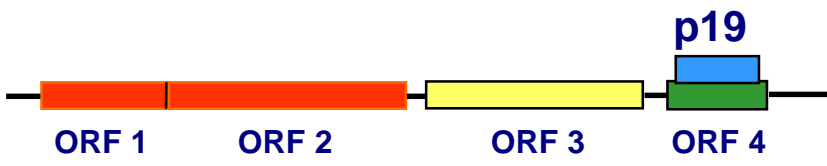


P19 molekuláris tolómérceként működik



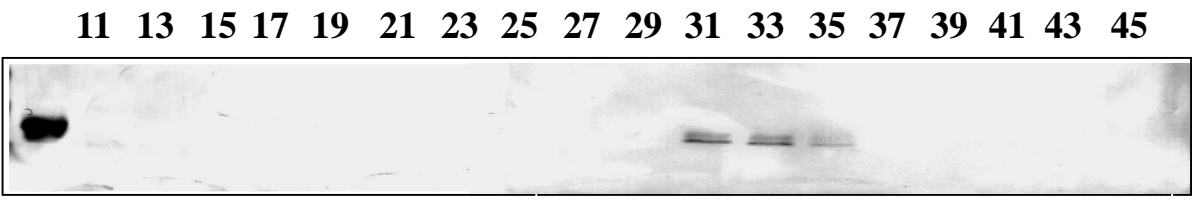
P19 működésének vizsgálata vírusfertőzött növényekben



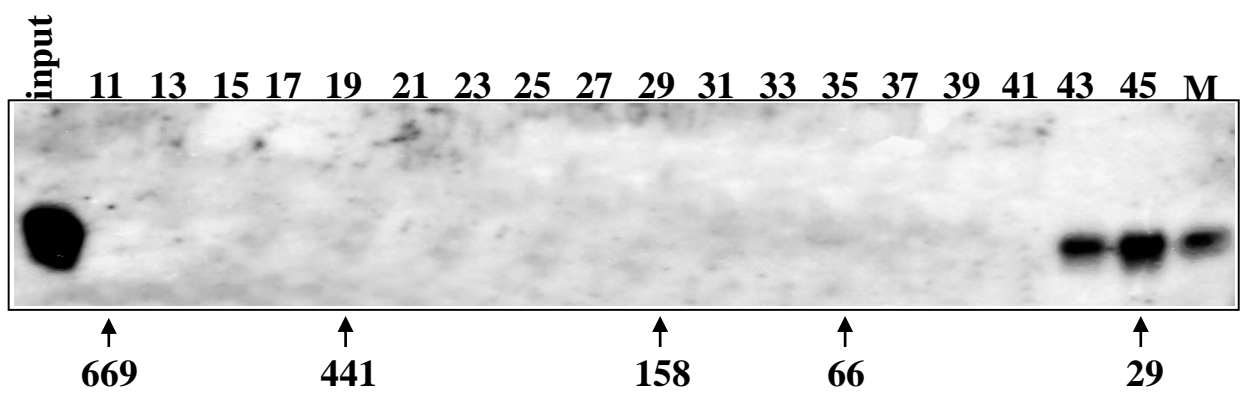


CymRSV
-21 nt siRNS

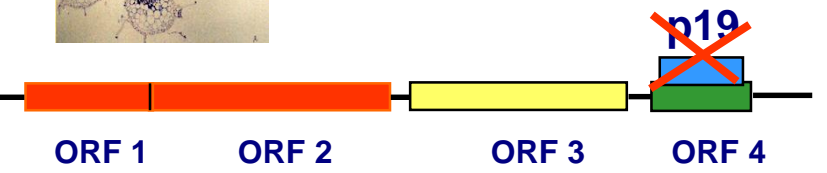
a-p19
Western



CymRSV
-28 kDa
-20 kDa



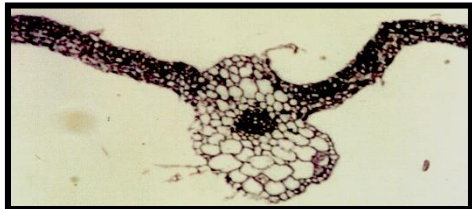
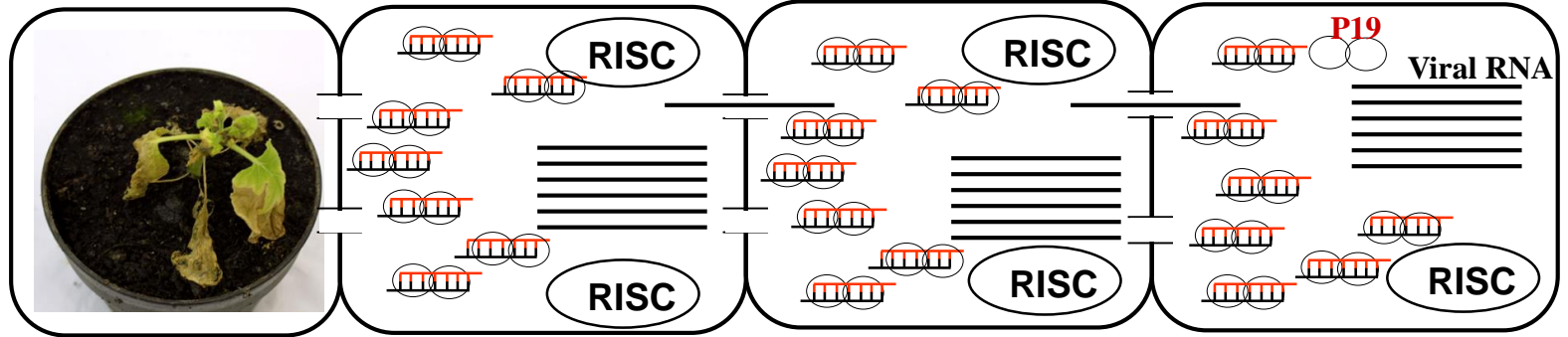
Cym19stop
-21 nt siRNS



Vírus fertőzés iránya

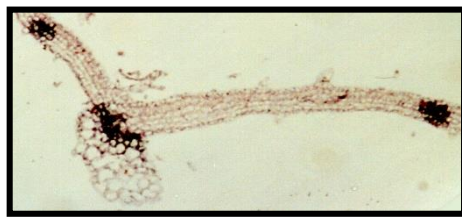
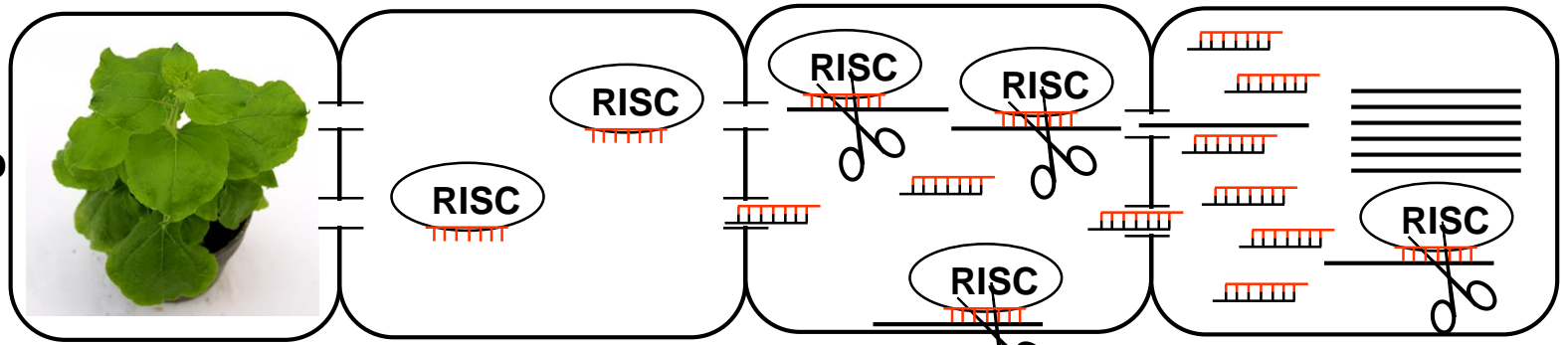


CymRSV



==== Virus RNS ○○ P19 dimer ㄣ Virus siRNS-ek

Cym19stop



==== Viral RNAs ○○ p19 dimers ㄣ Virus siRNAs

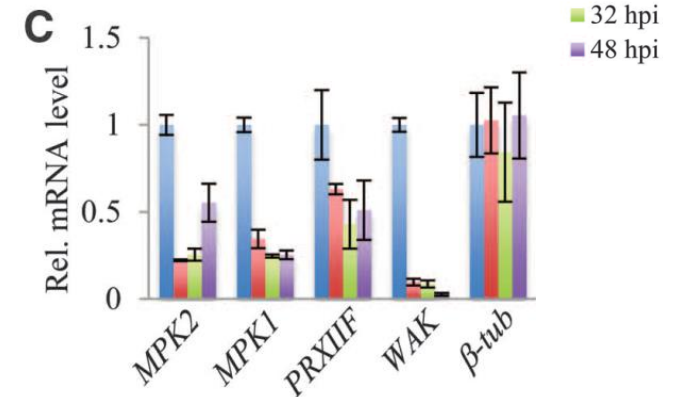
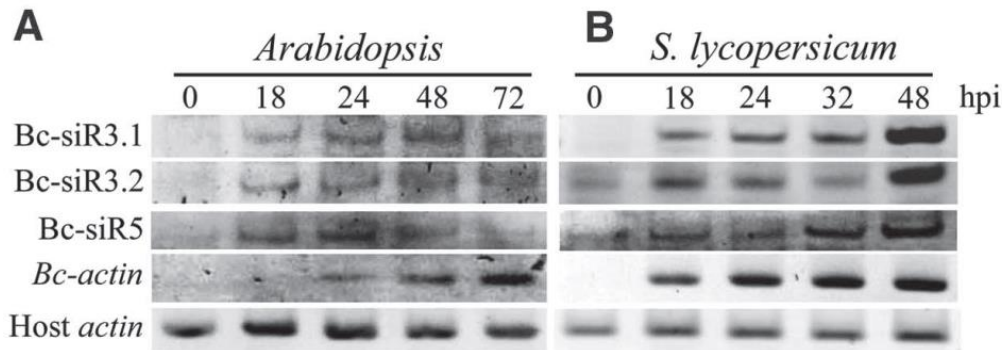
Botrytis cinerea

Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways

Botrytis cinerea fungal pathogene worldwide.

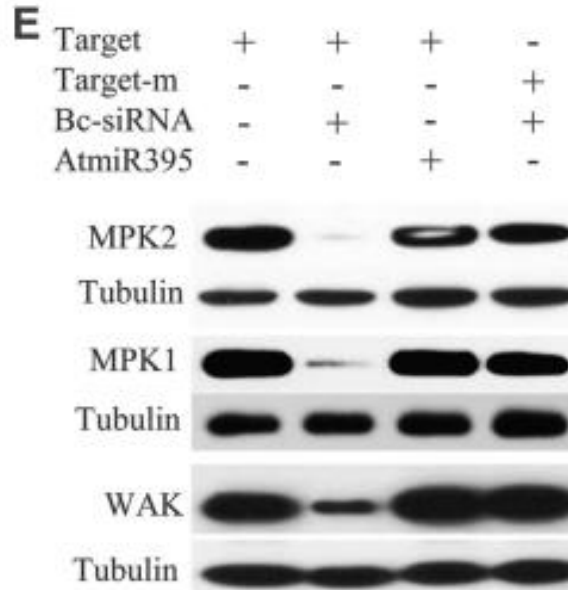
\$10 billion to \$100 billion in losses

sRNA libraries 73 Bc-sRNAs could target host genes in both *Arabidopsis* and *S. lycopersicum* under stringent target prediction criteria ,52 were derived from six retrotransposon long terminal repeats (LTR) loci in the *B. cinerea* genome, 13 were from intergenic regions of 10 loci, and eight were mapped to five protein-coding genes.

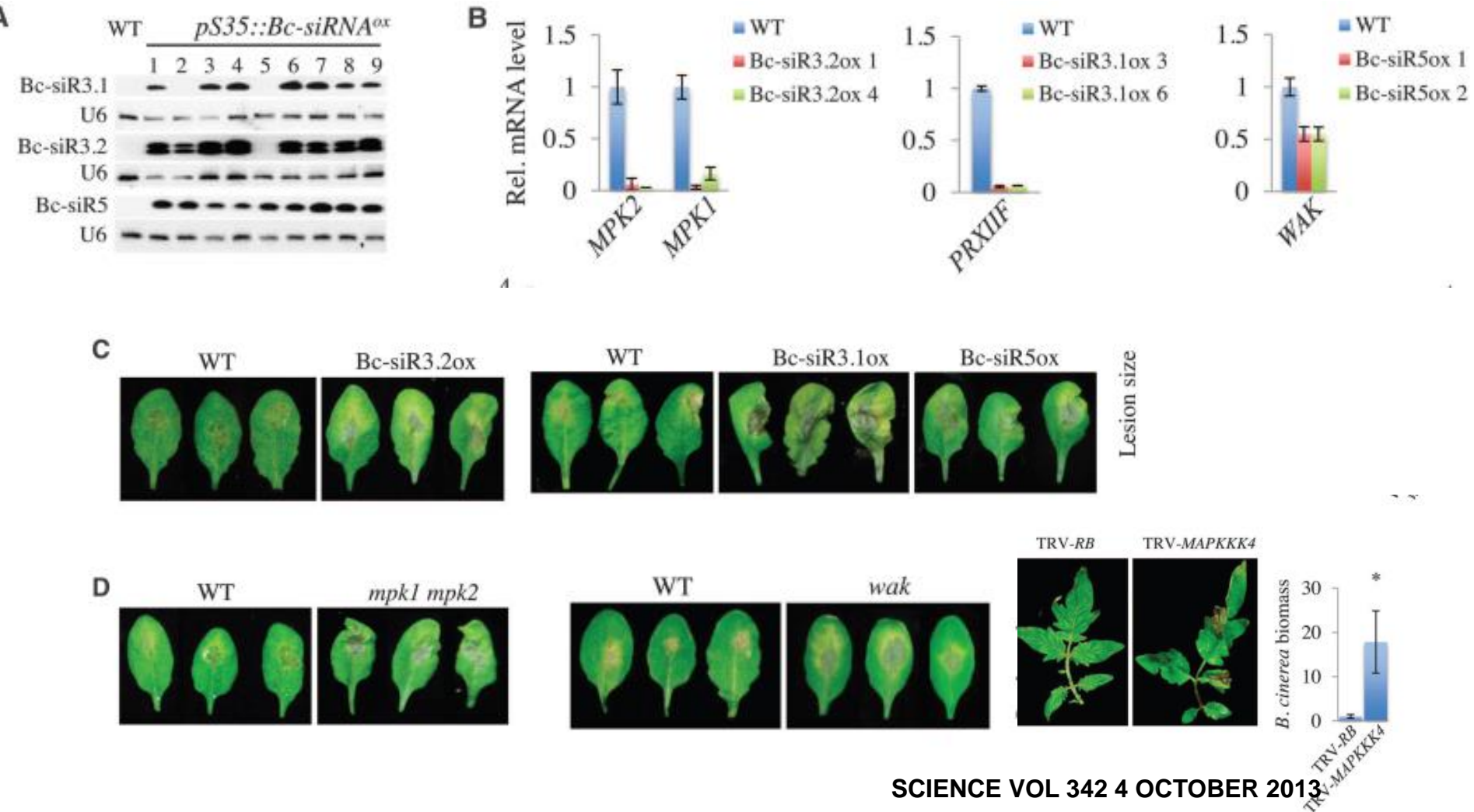


mitogen activated protein kinase, peroxiredoxin, cell wall-associated kinase important in defense reaction

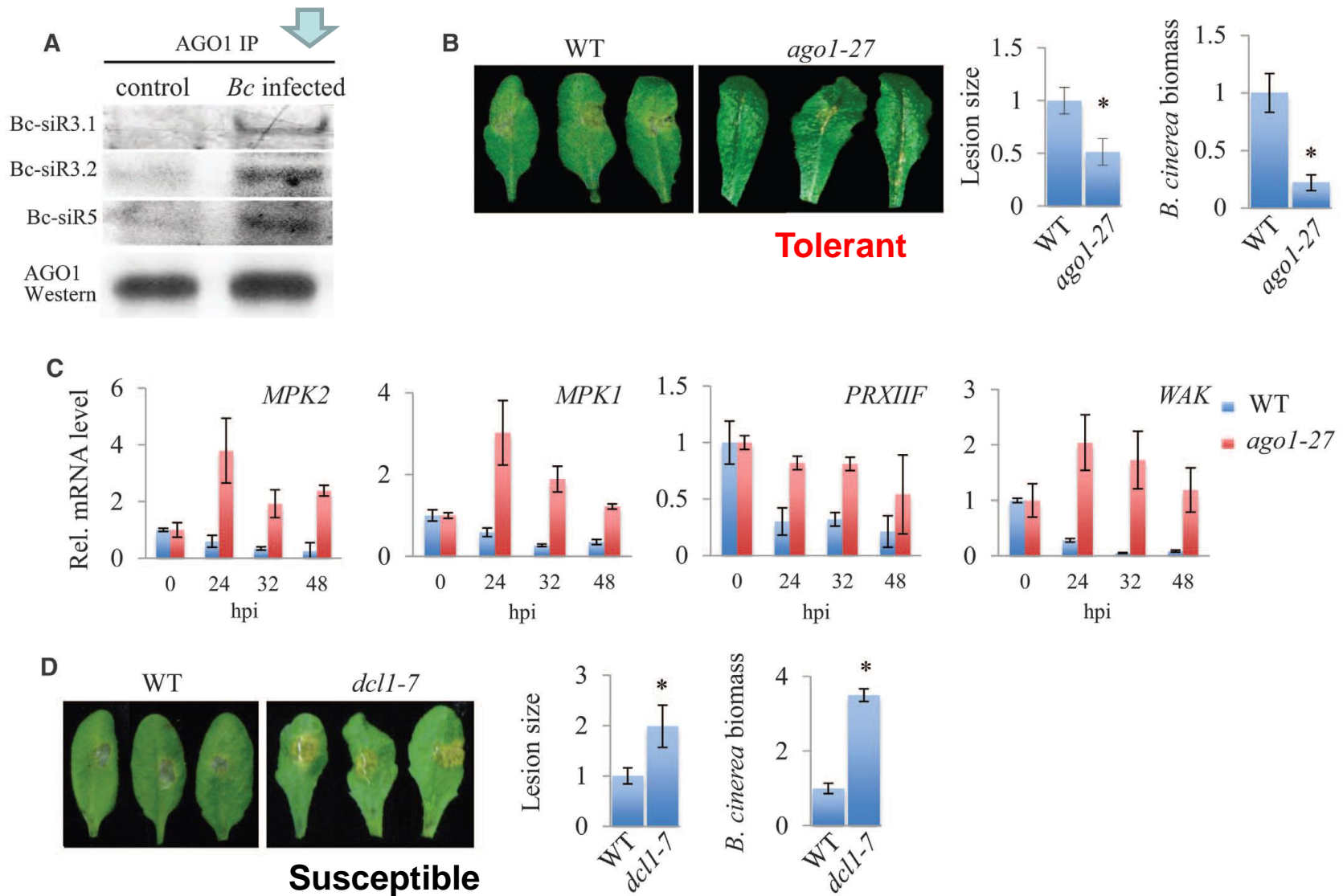
Coexpression of Bc-siR3.2 or Bc-siR5 with their host targets (HA-tagged) in *N. benthamiana*



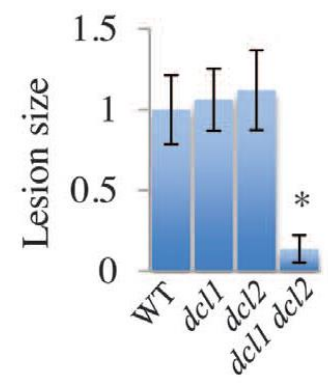
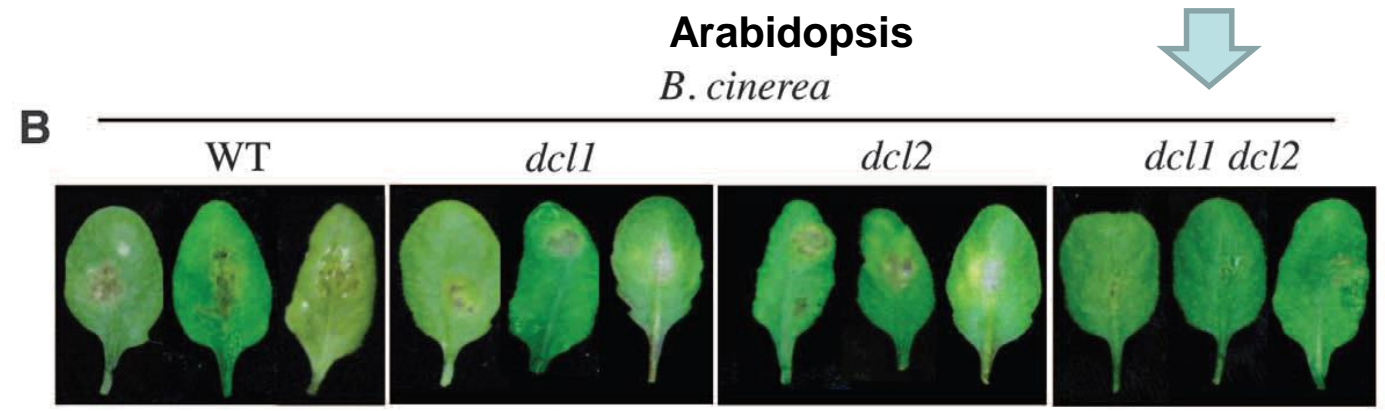
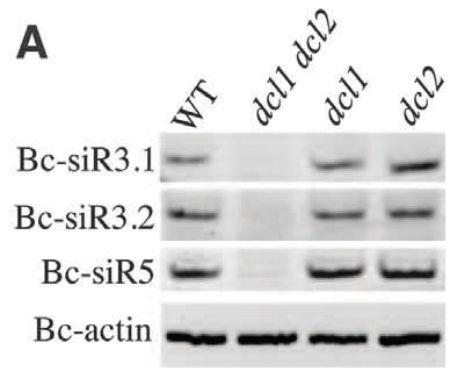
Expression of Bc-siR3.1, Bcsir3.2, or Bc-siR5 in transgenic Arabidopsis (artificial miRNA)



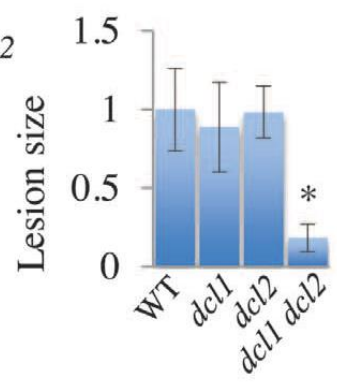
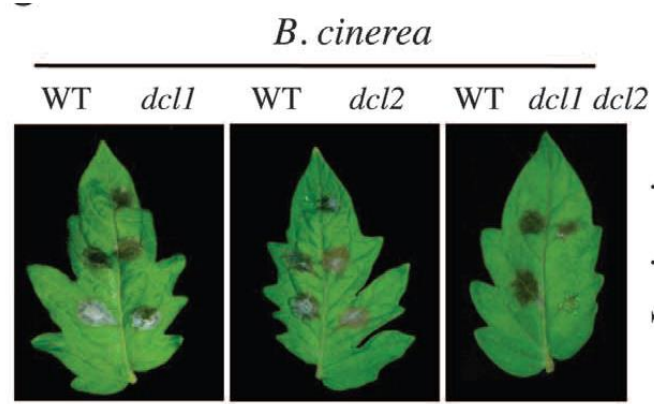
Bc-sRNAs hijack Arabidopsis AGO1 to suppress host immunity genes



B. cinerea dcl1 dcl2 double mutant is compromised in virulence



Tomato



Fejlődés biológiai folyamatok

Patogén elleni válaszok

Genom stabilitás fenntartása

EPIGENETIKA

(genetikai öröklődés *felett*)

A gének olyan öröklődési formájának vizsgálata (génszabályozás), mely nem jár együtt a DNS szekvenciájának megváltozásával.

DNS metiláció és a kromatin struktúra szabályozás

A genom 90 % átíródik RNS formába (humán és élesztő)

A

**legtöbb RNS termék nem kódoló régiókról származik
(Heterokromatin)**

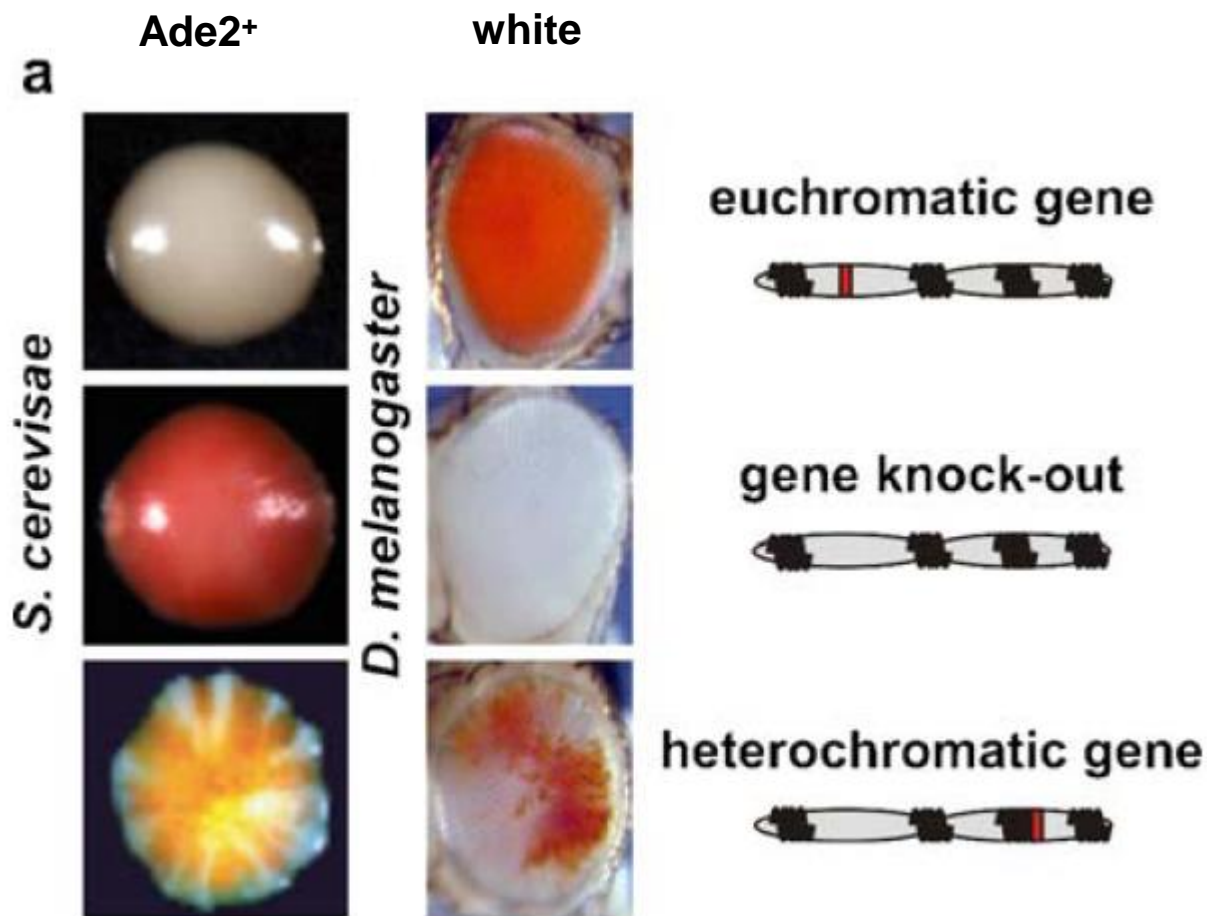
**Nem kódoló kis RNS-ek fontos szerepet játszanak a
kromatin szerkezet kialakításában és fenntartásában.**

Eukromatin transzkripcionálisan aktív

Heterokromatin nem elérhető DNS kötő faktorok számára



transzkripcionálisan inaktív

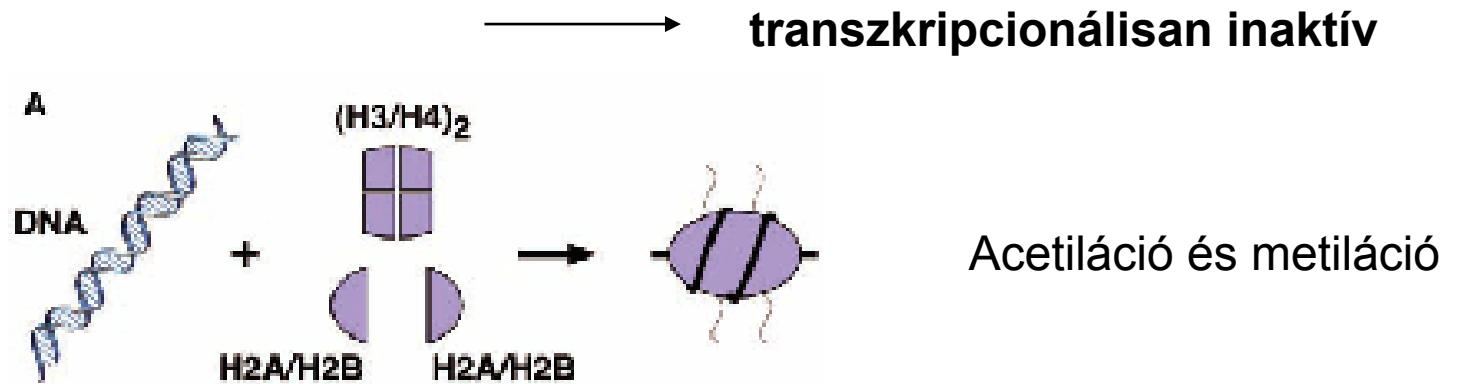


variegating phenotype in a clonal population of cells (position effect variegation, PEV).

RNAi és a genom - heterokromatinizáció

Eukromatin transzkripcionálisan aktív

Heterokromatin nem elérhető DNS kötő faktorok számára



Euchromatin : methylation of lysine4 (H3K4me) and acetylation of lysine 9 (H3K9ac)

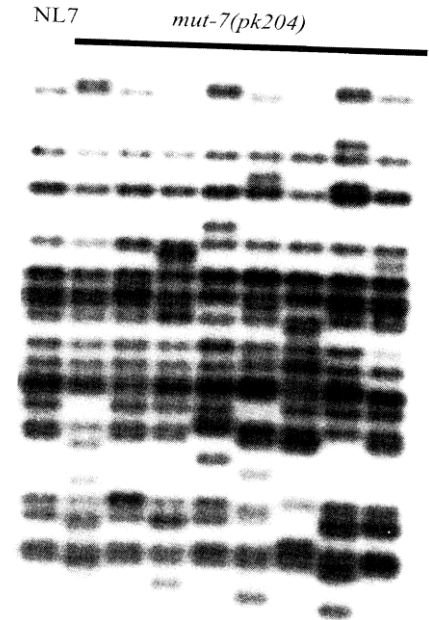
Heterochromatin : methylation of lysine 9 (H3K9me)

Genom stabilitás fenntartása

Caenorhabditis elegans
mut-7 silencing mutáns

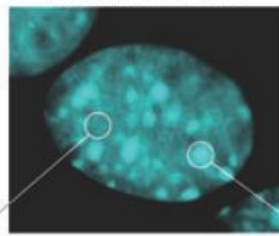
Retrotranszpozonok a genom → 40% humán
50-80% egyes fűfélék

Transzpozon aktiváció RNS silencing
mutánsokban



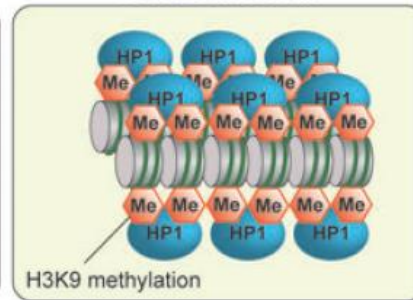
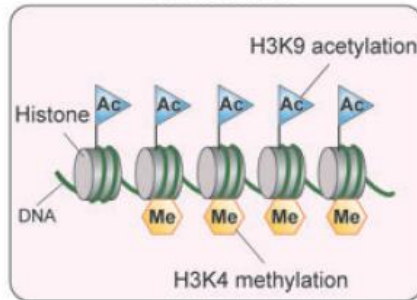
siRNS-ek a Tc1 transzpozonról → RNA silencing ellenőrzés

(A) Mammalian cells

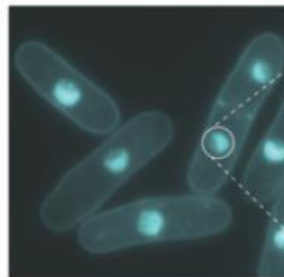


Euchromatin

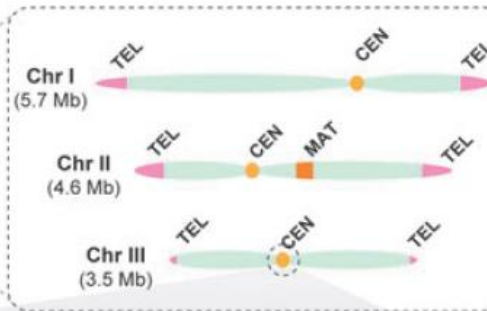
Heterochromatin



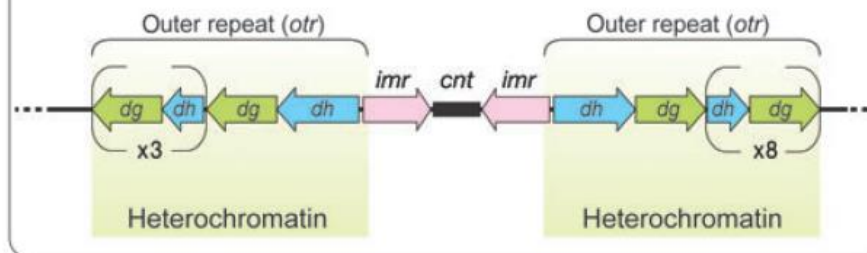
(B)



Schizosaccharomyces pombe

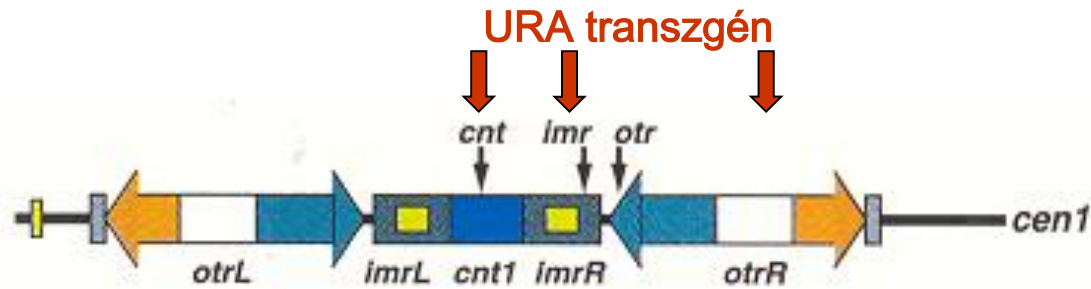


cen 3 (~110 kb)



RNAi rendszer kell H3K9 metilációhoz és centroméra heterokromatin szerkezet fenntartásához

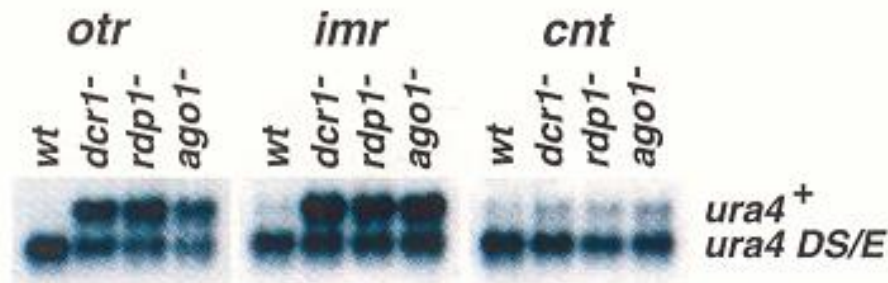
Élesztő



A centromérikus heterokromatin szerkezete

Dcr1- DICER K.O.
Rdp1- RdRP K.O.
Ago1- AGO K.O:

Szükségesek az RNAi működéshez



RNAi nélkül az inaktív URA transzgén aktiválódik



Heterokromatin szerkezet felbomlik

DNS metiláció növényekben

- **CG->MET1**

tartja fenn, homológ az emlősben leírttal.

- **CHG->CMT3**

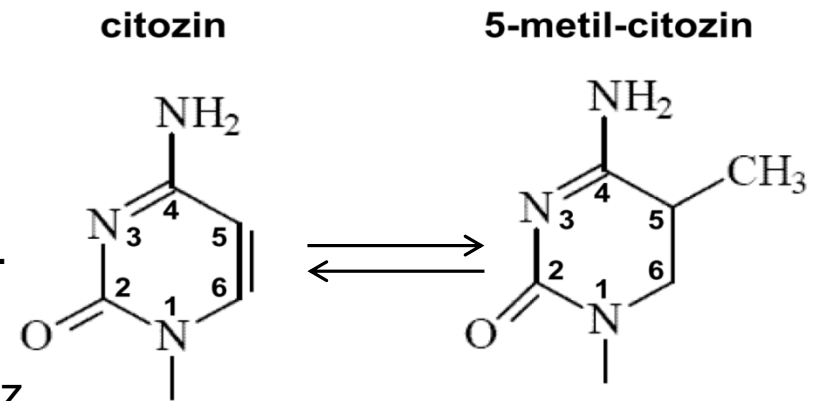
tartja fenn, ami a H3 Lys 9 met2-t ismeri fel.

- **CHH->DRM2**

tartja fenn, ami kisRNS függő metilációt tesz lehetővé

Kis RNS-függő DNS metiláció

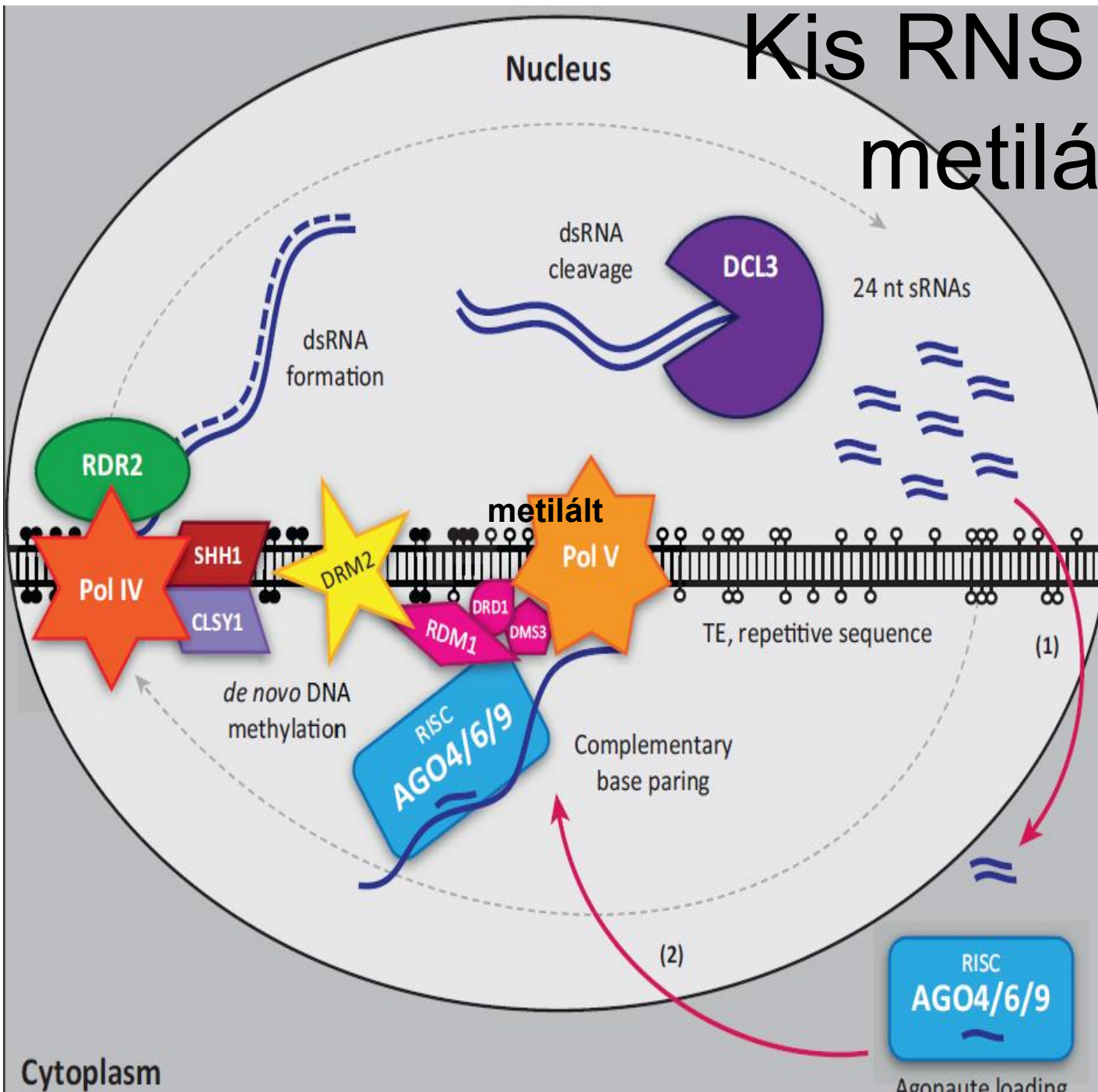
1. Létesítés
2. Fenntartás



A legtöbb esetben transzpozon elemekben vagy azok közelében metilált. Ez alapján feltételezik, hogy elsődleges funkciója az ilyen elemek inaktíválása.

Transcriptional gene silencing TGS

Kis RNS alapú metiláció



Loss of *Karma* transposon methylation underlies the mantled somaclonal variant of oil palm

Meilina Ong-Abdullah¹, Jared M. Ordway², Nan Jiang², Siew-Eng Ooi¹, Sau-Yee Kok¹, Norashikin Sarpan¹, Nuraziyan Azimi¹, Ahmad Tarmizi Hashim¹, Zamzuri Ishak¹, Samsul Kamal Rosli¹, Fadila Ahmad Malike¹, Nor Azwani Abu Bakar¹, Marhalil Marjuni¹, Norziha Abdullah¹, Zulkifli Yaakub¹, Mohd Din Amiruddin¹, Rajanaidu Nookiah¹, Rajinder Singh¹, Eng-Ti Leslie Low¹, Kuang-Lim Chan¹, Norazah Azizi¹, Steven W. Smith², Blaire Bacher², Muhammad A. Budiman², Andrew Van Brunt², Corey Wischmeyer², Melissa Beil², Michael Hogan^{2†}, Nathan Lakey², Chin-Ching Lim³, Xaviar Arulandoo³, Choo-Kien Wong⁴, Chin-Nee Choo⁴, Wei-Chee Wong⁴, Yen-Yen Kwan⁵, Sharifah Shahrul Rabiah Syed Alwee⁵, Ravigadevi Sambanthamurthi¹ & Robert A. Martienssen⁶



Director of the Advanced Biotechnology and Breeding Centre of the Malaysian Palm Oil Board

Oil Palm

Common Name	:	African oil palm
Genus	:	Elaeis
Species	:	guineensis
Abbreviation	:	E. guineensis
Origin	:	Africa

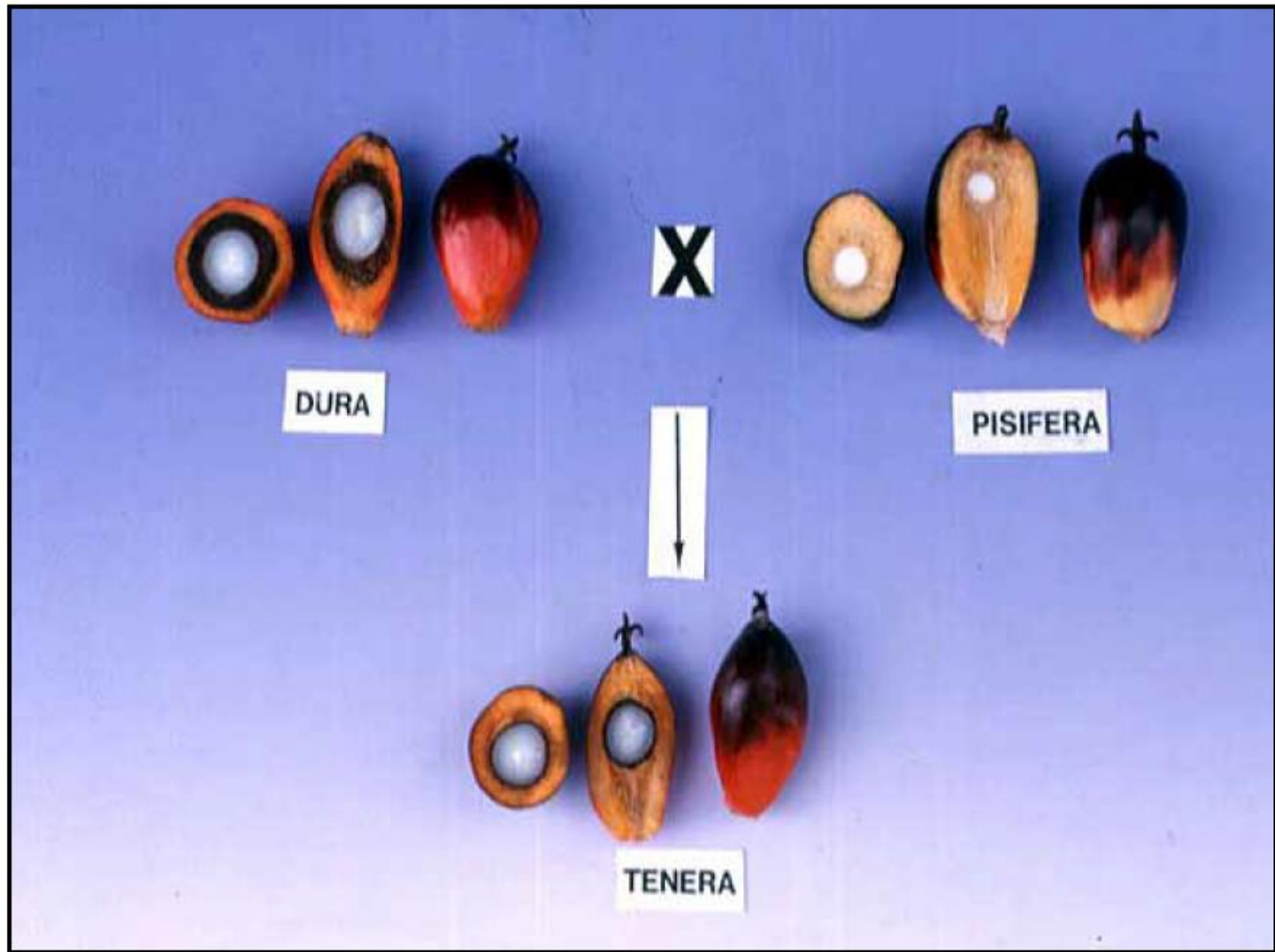


- **Oil palm is unique in that it produces oil from the mesocarp and kernel .**
- **The first oil palm was introduced into South East Asia and planted in Bogor Botanical Gardens, Java in 1848.**
- **The productivity of oil palm is unprecedented, producing ~37% of the total vegetable oil on only 5% of the total acreage of vegetable oil crops.**



Oil palm hybrids have 30 % more oils

Sh Sh



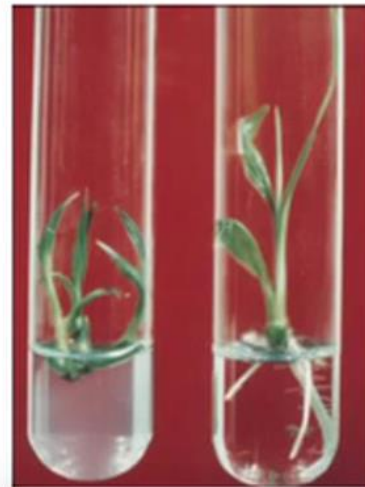
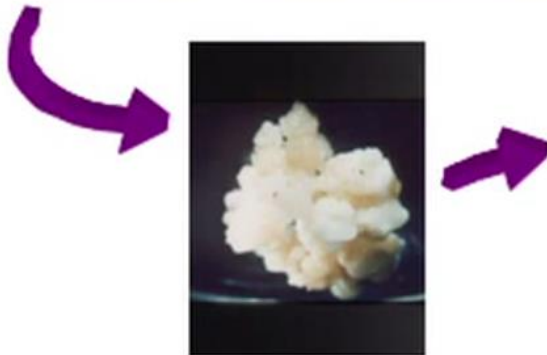
sh sh

Sh sh

Why are clones successful?

Clones maintain HETEROZYGOSITY – maintain the contribution of both parents for every gene (hybrid vigor).

Cloning oil palm trees

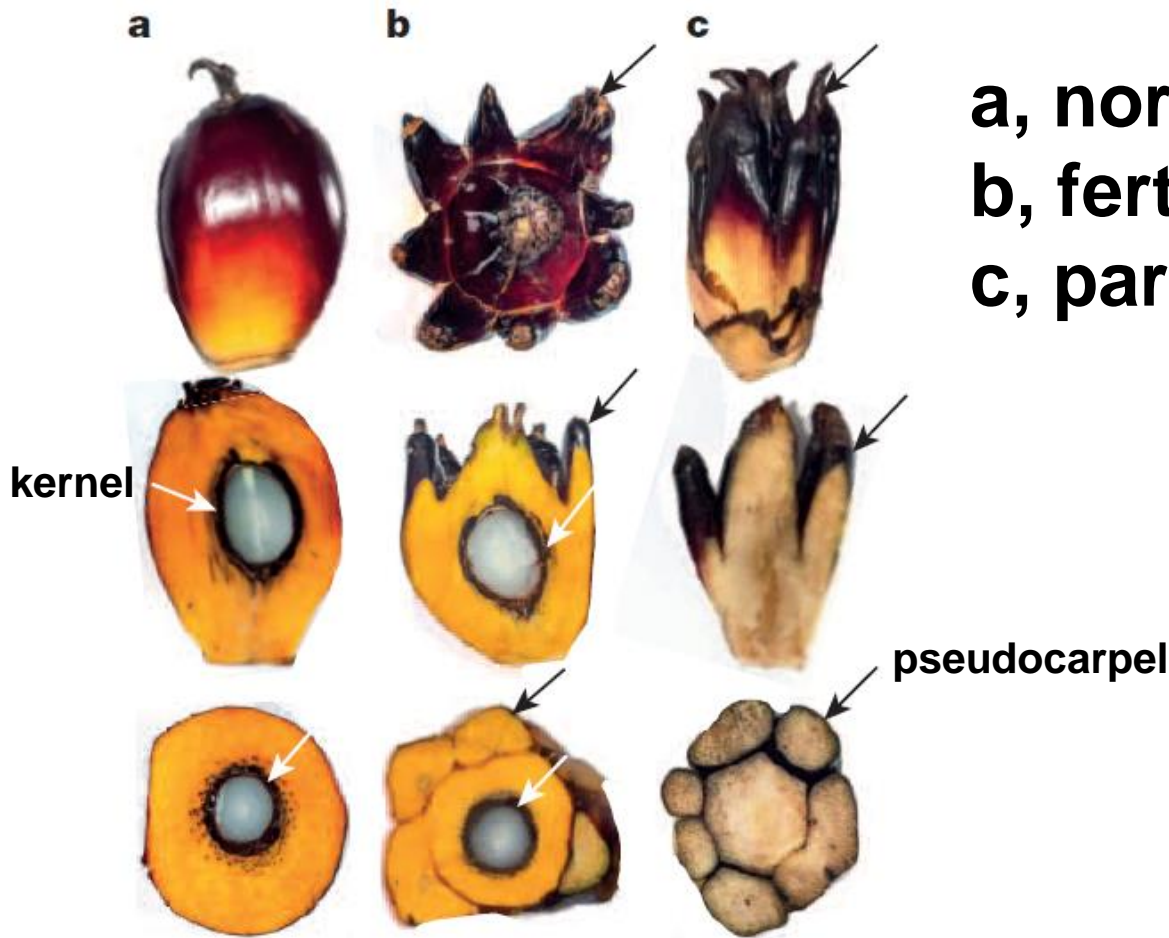


Cell culture



- 1970s: successful development of oil palm tissue culture
- mass propagation of 1000s of uniform elite clones with the potential to increase oil yield by 30%.
- mid 1980s: the emergence of clonal abnormality

anism responsible remains unknown¹. The oil palm fruit 'mantled' abnormality is a somaclonal variant arising from tissue culture that drastically reduces yield, and has largely halted efforts to clone elite hybrids for oil production²⁻⁴. Widely regarded as an epi-



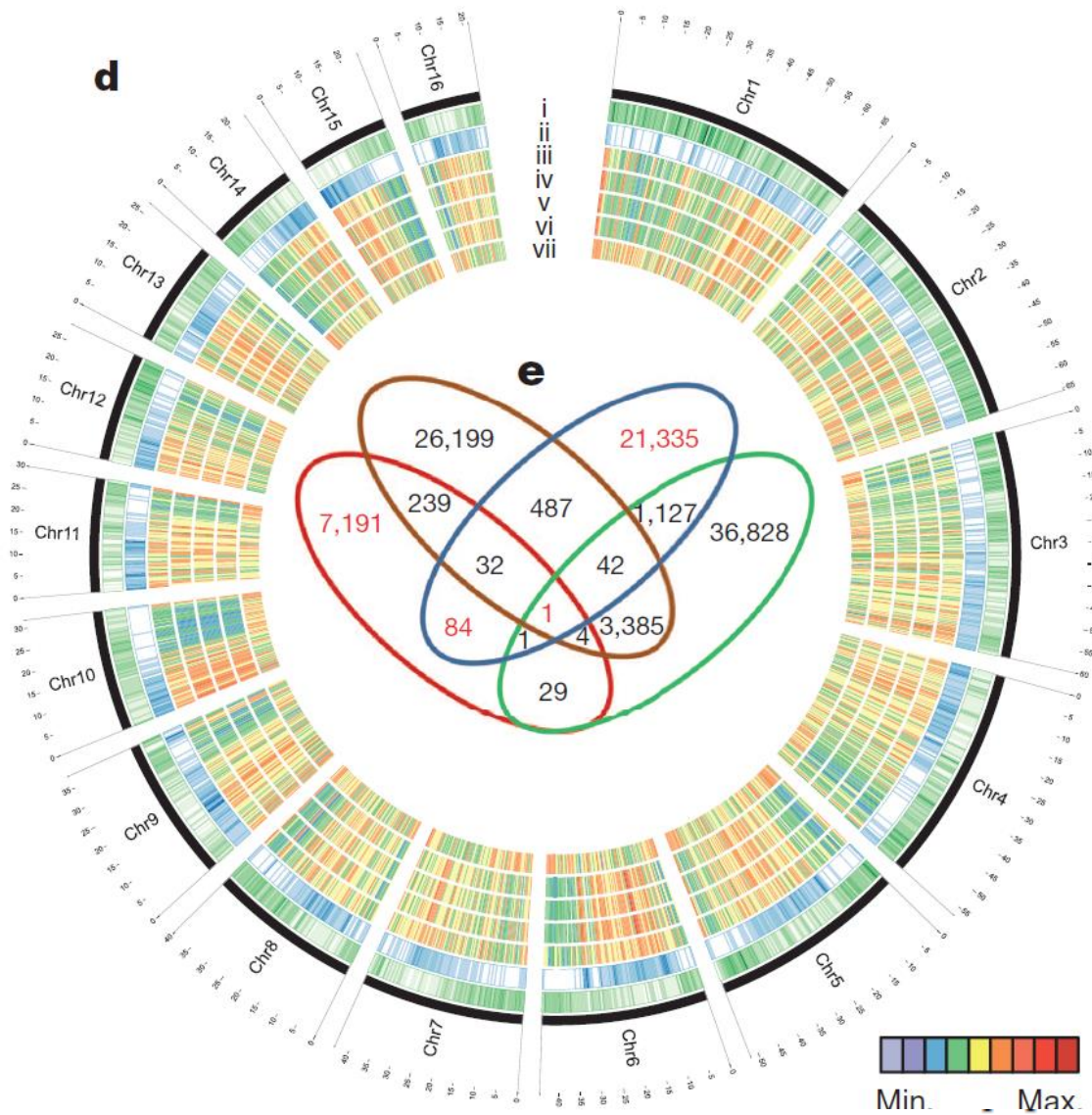
a, normal

b, fertile mantled

c, parthenocarpic mantle

Genome-wide DNA methylation analysis

To maximize genotypic diversity, 15 independent somaclonal lineages were used from 4 independent sources.



Circos plot of oil palm chromosomes

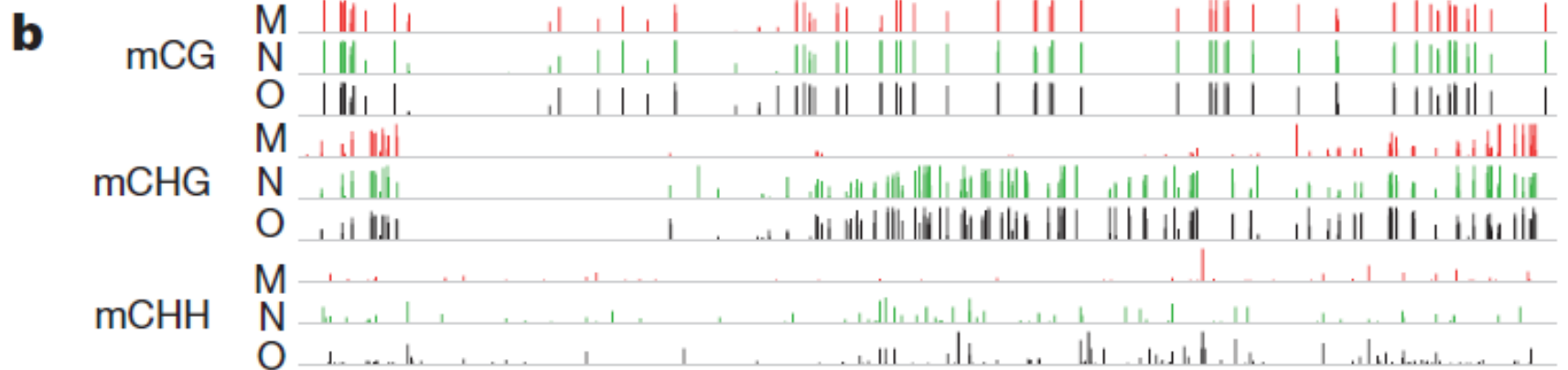
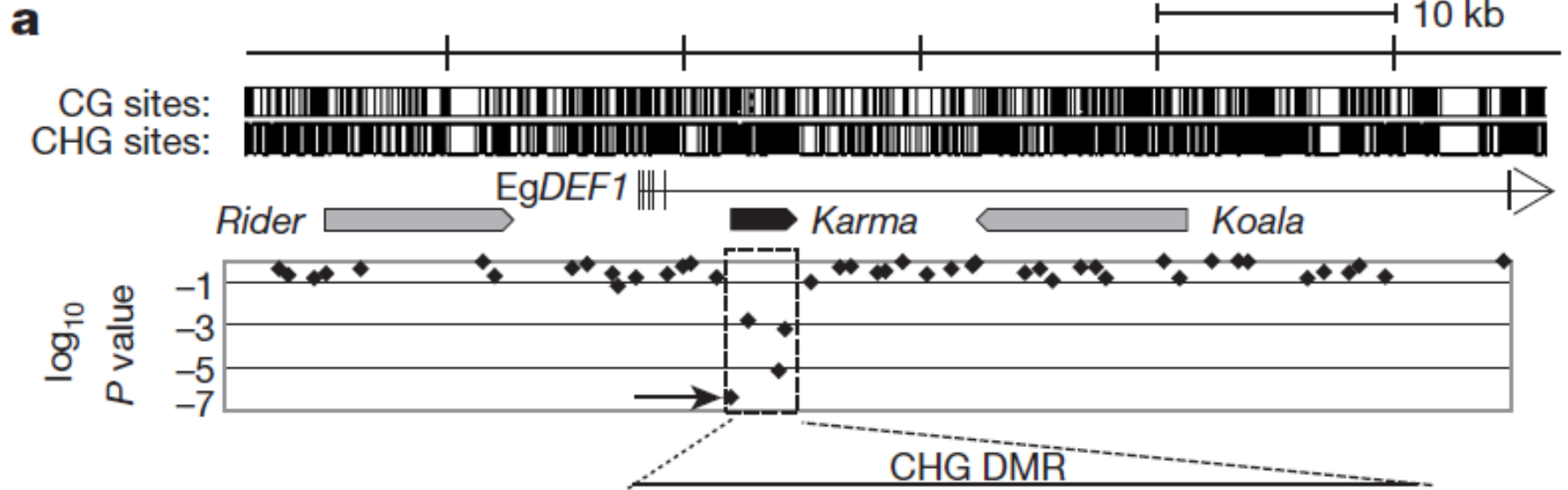
ation in epigenetic response to tissue culture. A single microarray feature detected differential methylation between normal and mantled clones in all four populations (Fig. 1e). This feature lies within the ~35 kb intron 5 of *EgDEF1* (Fig. 2a), the oil palm orthologue of the

DEF1 is an Arabidopsis AP3 orthologue

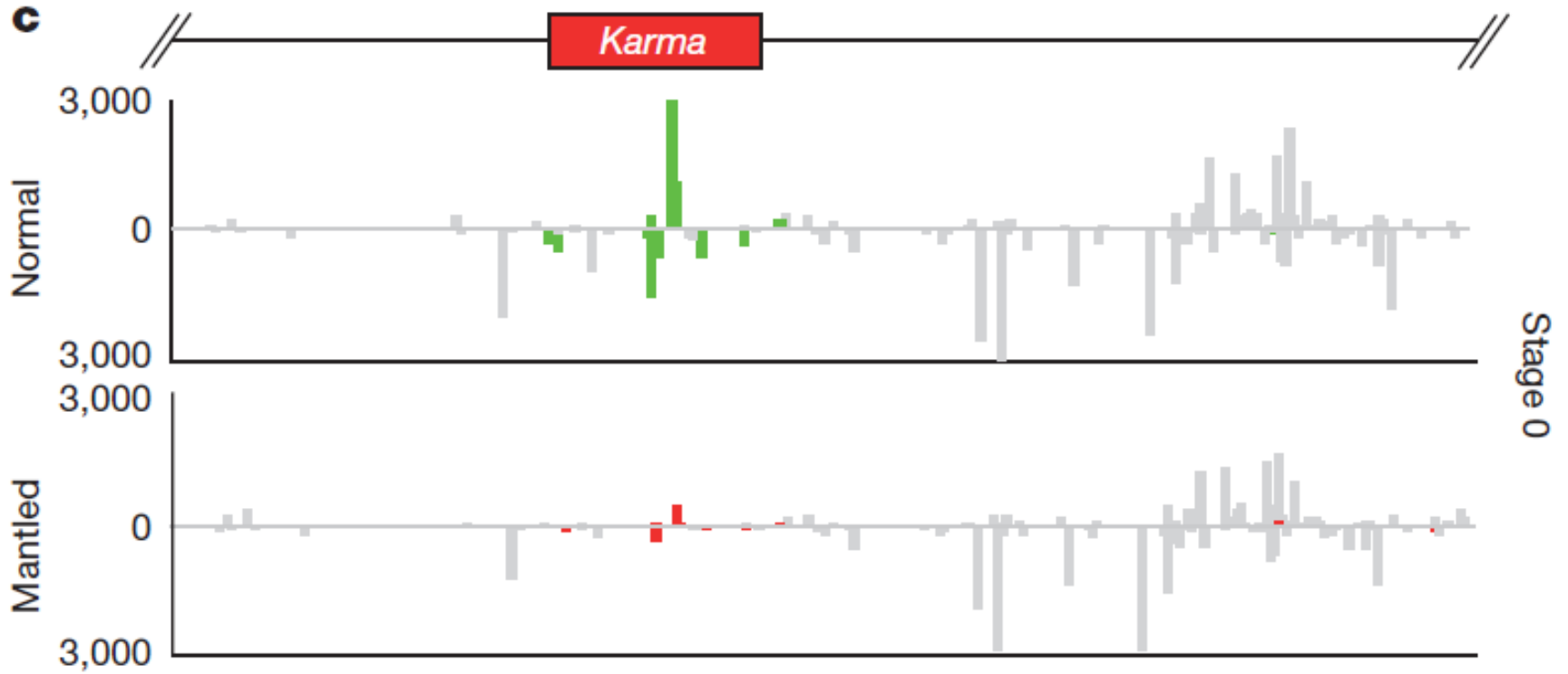
apetala3 mutant



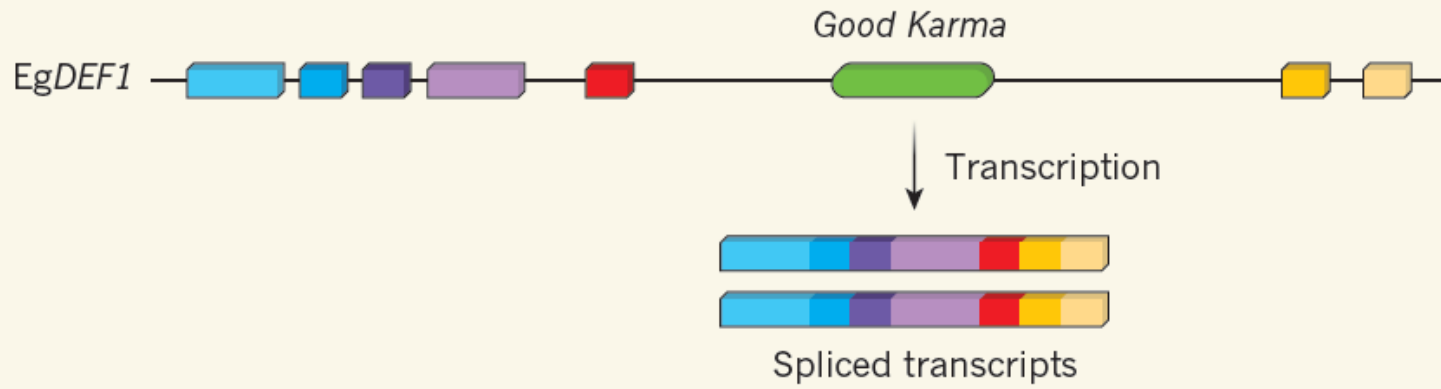
Hypomethylation of Karma associated with the mantlad phenotype



24 nt siRNAs lost from Karma in mantled shoots



a



b

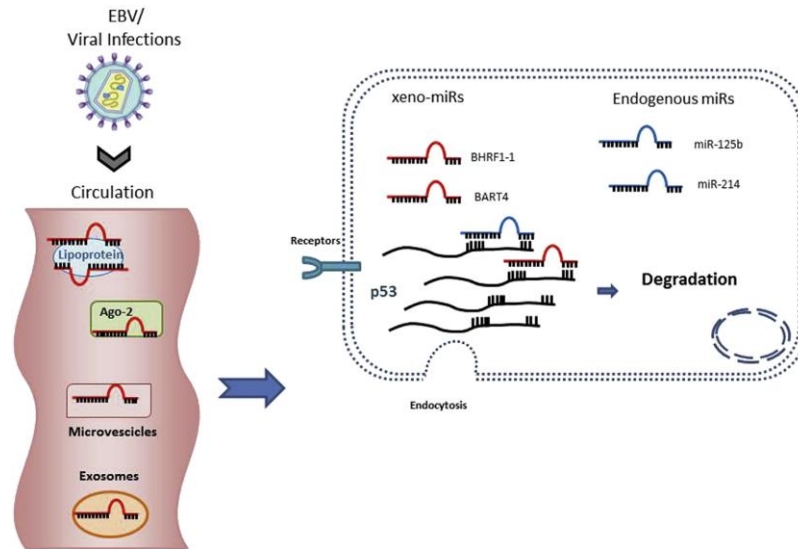


Könnyű azonosítás

Circulating free xeno-microRNAs

Table 1 – Circulating-free xeno-microRNAs in human plasma.

Xeno-miRNAs	Putative target	Disease	Reference
Rice miR-168a	LDLRAP1	Atherosclerosis	(Zhang et al., 2012a, 2012b)
KSHV-miR-K12-11	C/EBPβ	Lymphoma	(Boss et al., 2011)
KSHV-miR-12-7	MICB	Glioblastoma	(Herman et al., 2015)
miR-K-12-10b, miR-K-12-12*	Agonist TLR8	Sepsis	(Tudor et al., 2014)
Cow miR-29	HDAC4, TGFbeta3	Osteoclast differentiation	(Baier et al., 2014)
BHRF1-1 miRNA	p53	CLL	(Ferrajoli et al., 2015)
miR-BART16-1	LMP1	HLH	(Zhou et al., 2015)
miR-BART3, miR-BART7	Unknown	NPC	(Zhang et al., 2015)



Zhang, L., Hou, D., Chen, X., Li, D., Zhu, L., Zhang, Y., Li, J.,
Bian, Z.,
Liang, X., Cai, X., et al., 2012a. Exogenous plant MIR168a
specifically targets mammalian LDLRAP1: evidence of
crosskingdom
regulation by microRNA. *Cell Res.* 22, 107e126.

The authors found **rice miR-168a** in sera of Chinese subjects, demonstrating how it regulates low-density **lipoprotein receptor adapter protein 1** (LDLRAP1), a gene involved in cholesterol metabolism, influencing LDL removal from plasma, at least in a mouse model. These data were not reproduced by a subsequent

Methylation of plant miRNAs influences negatively the qRT-PCR, and is considered a reason for they underrepresentation in screening experiments ([Yu et al., 2005](#)), but could also be a **way for selective absorption in the gut**.

Cross-kingdom inhibition of breast cancer growth by plant miR159

Andrew R Chin^{1,6}, Miranda Y Fong¹, George Somlo², Jun Wu³, Piotr Swiderski⁴, Xiwei Wu⁵, Shizhen Emily Wang¹

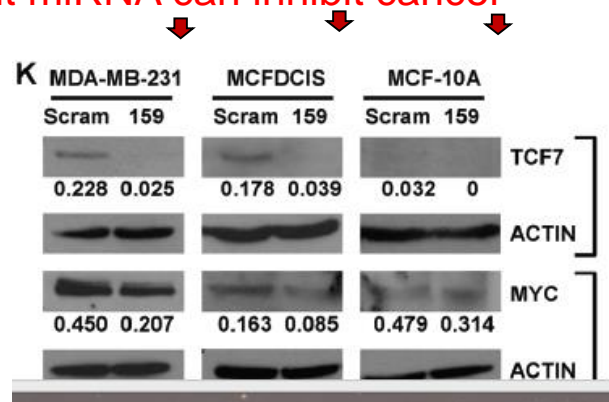
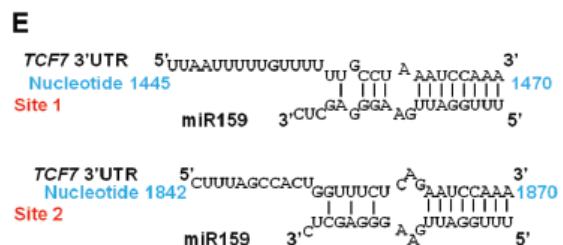
¹Department of Cancer Biology, ²Department of Medical Oncology, ³Department of Molecular Medicine, ⁴Cores of Synthetic and Biopolymer Chemistry, ⁵Cores of Integrative Genomics, City of Hope Beckman Research Institute and Medical Center, Duarte, CA 91010, USA; ⁶City of Hope Irell & Manella Graduate School of Biological Sciences, Duarte, CA 91010, USA

plant miRNA miR159 abundance in the serum was inversely correlated with breast cancer incidence.

In breast cancer cells, a mimic of miR159 was capable of inhibiting proliferation by **targeting TCF7** (Wnt signaling transcription factor), leading to a decrease in MYC protein levels.

Oral administration of **miR159** mimic significantly **suppressed** the growth of xenograft breast tumors in mice.

These results demonstrate for the first time that a plant miRNA can inhibit cancer growth in mammals.

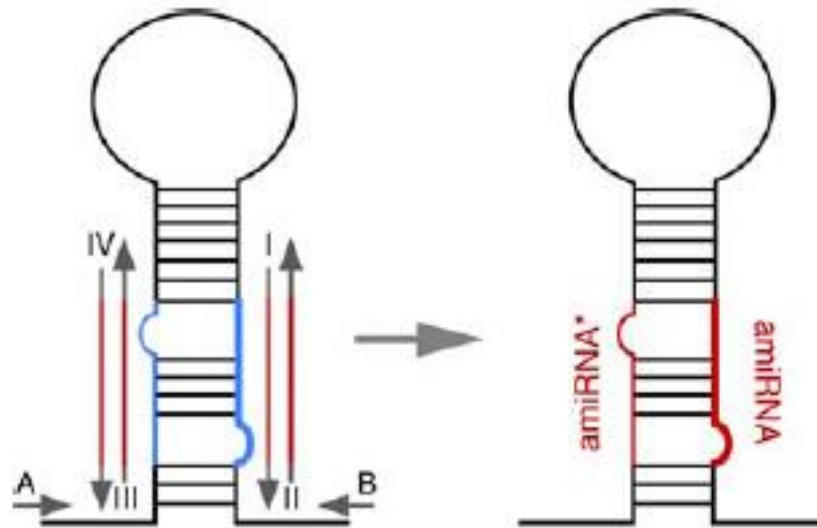


breast cell lines

Felhasználás

Mesterséges miRNS

1. Nem hőérzékeny
2. Nagyon specifikus



TECHNIQUES FOR MOLECULAR ANALYSIS

Gene silencing in plants using artificial microRNAs and other small RNAs

Stephan Ossowski[†], Rebecca Schwab^{†,*} and Detlef Weigel^{*}

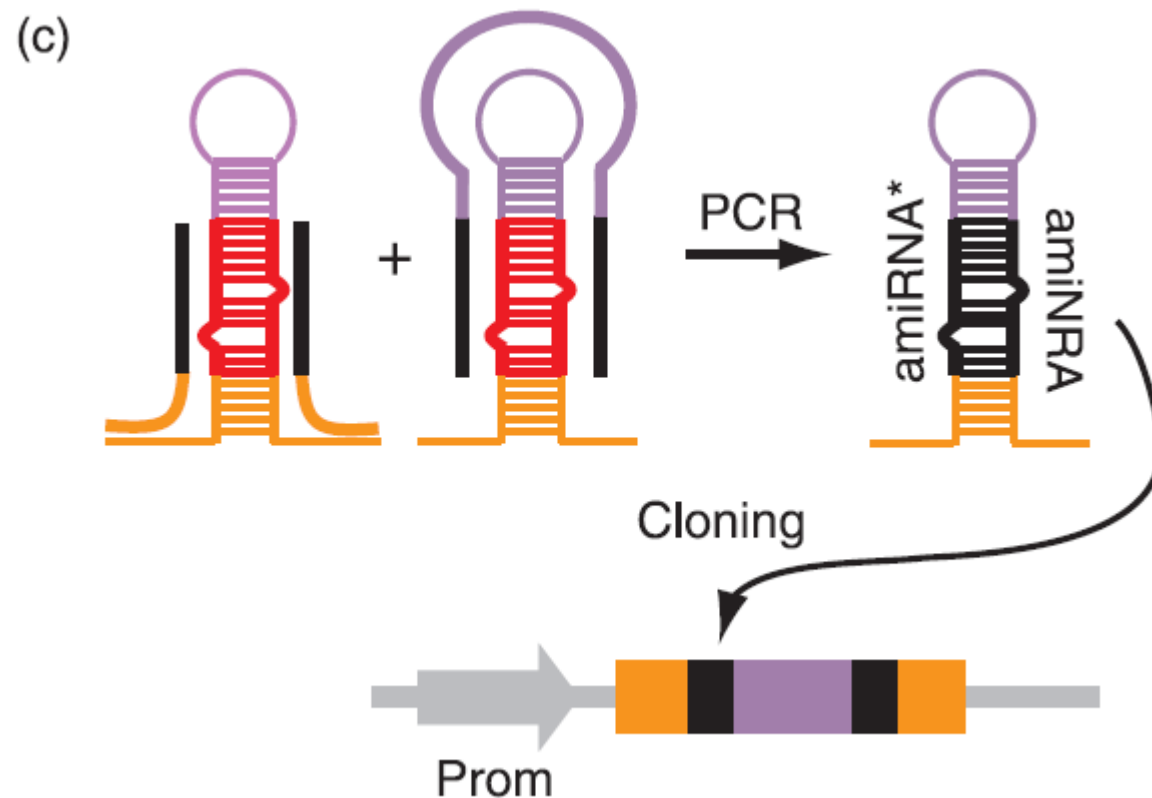
Department of Molecular Biology, Max Planck Institute for Developmental Biology, 72076 Tübingen, Germany

Received 8 May 2007; revised 10 September 2007; accepted 17 September 2007.

*For correspondence (fax +49 7071 601 1412; e-mail weigel@weigelworld.org).

[†]These authors contributed equally to this work.

[‡]Present address: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA.



wt

Mutant

amiRNA



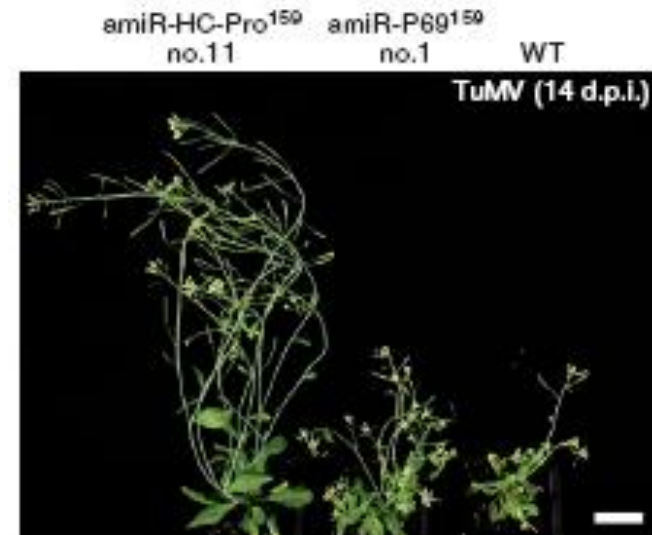
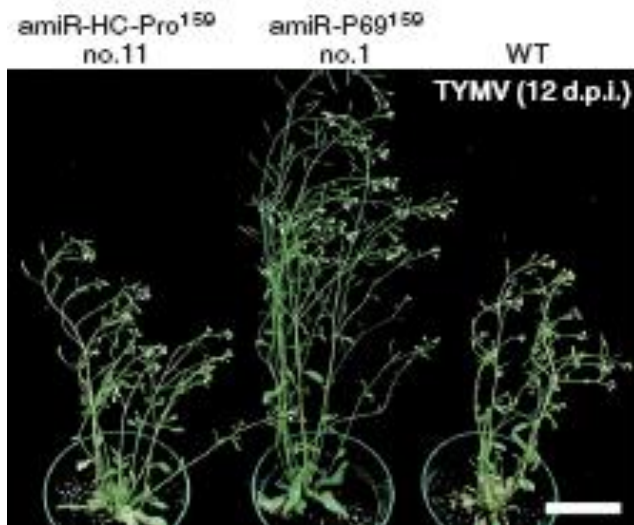
amiR-trichome targets MYB genes, CAPRICE (CPC),
TRIPTYCHON (TRY) trichome patterning.

Schwab et al. 2006

Expression of artificial microRNAs in transgenic *Arabidopsis thaliana* confers virus resistance

nature
biotechnology

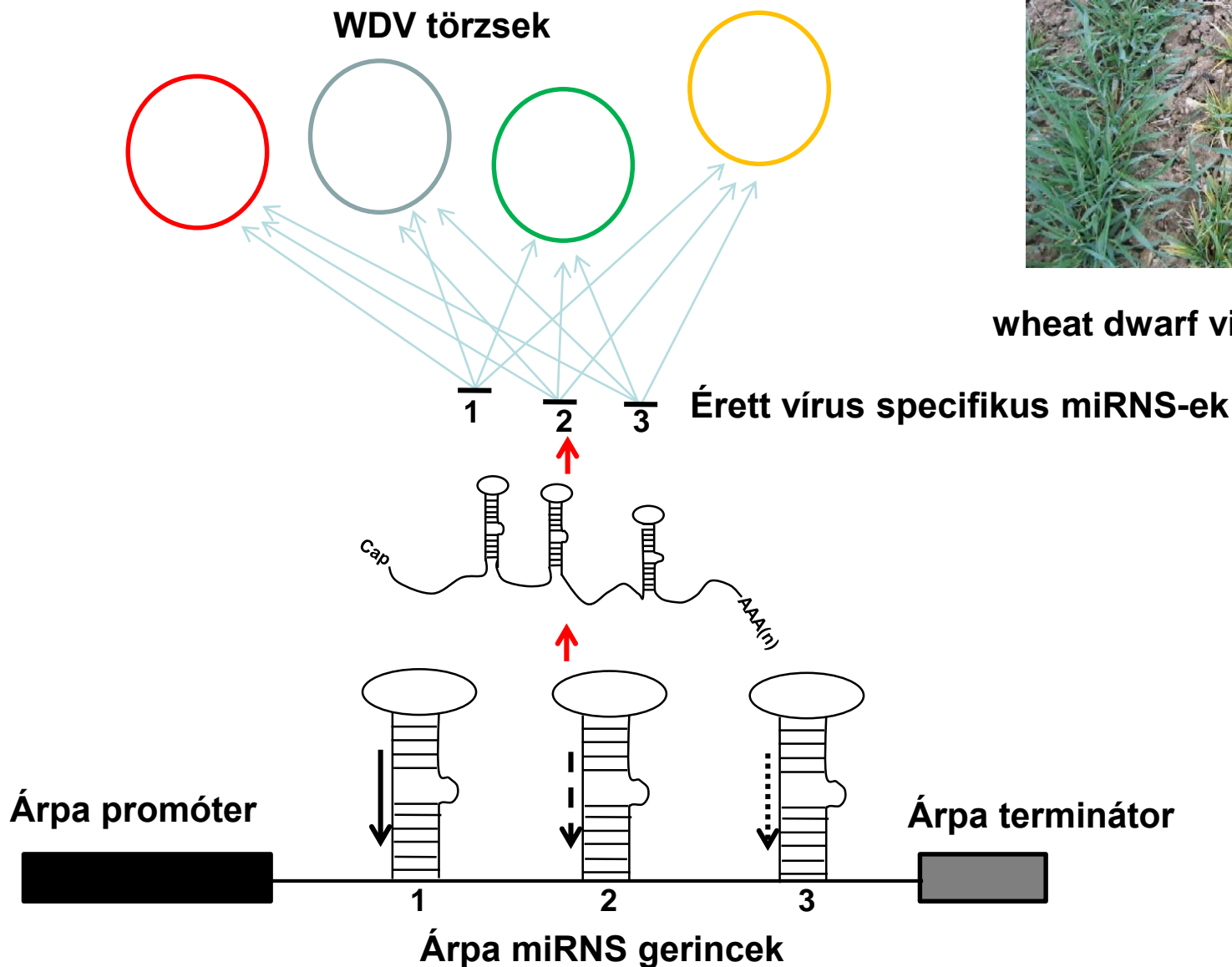
Qi-Wen Niu^{1,4}, Shih-Shun Lin^{1,4}, Jose Luis Reyes^{1,3}, Kuan-Chun Chen^{1,3}, Hui-Wen Wu¹,
Shyi-Dong Yeh² & Nam-Hai Chua¹



Búzatörpülés vírus rezisztens árpa előállítása mesterséges miRNS segítségével



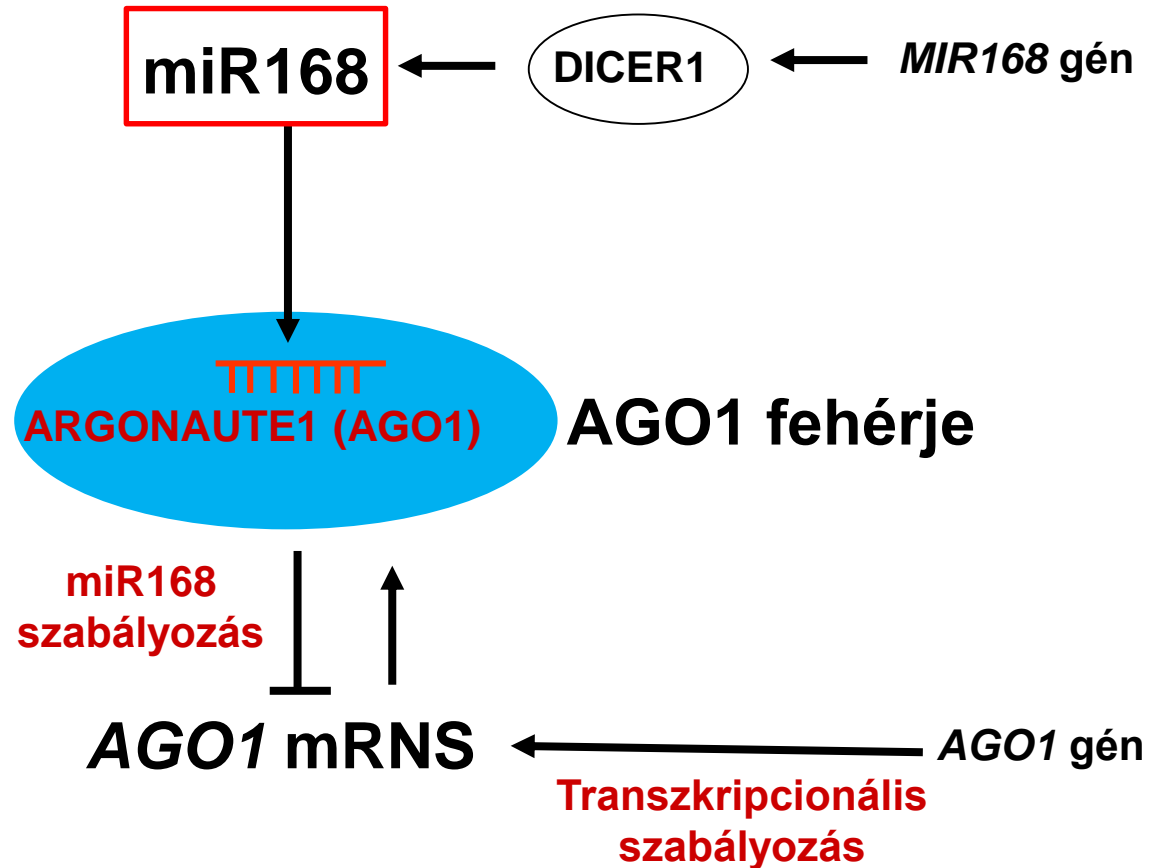
wheat dwarf virus (WDV)



Második trükk

ARGONAUTE1 (AGO1)

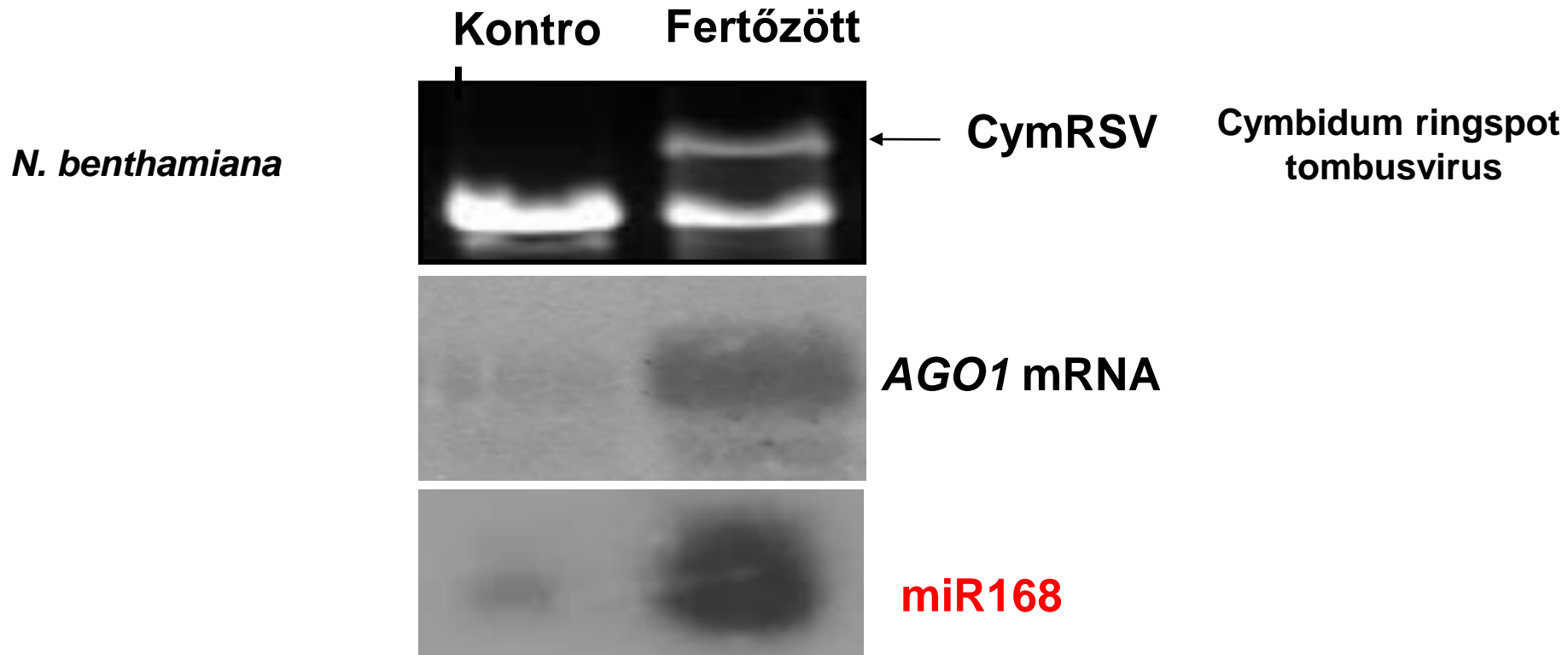
Az RNS csendesítés központi végrehajtó molekulája



**Több, az utóbbi években megjelent közlemény
bemutatta, hogy vírusfertőzött növényekben az
AGO1 mRNS és a miR168 szintje megemelkedett.**

(Csorba et al, 2007; Havelda et al, 2008; Zhang et al, 2006....)

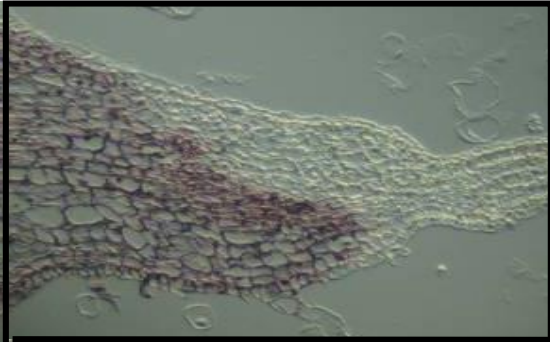
AGO1 mRNS és miR168 együttes indukciója vírusfertőzött növényekben



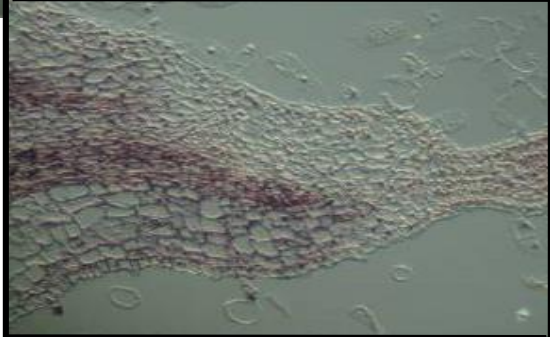
N. benthamiana

**A megemelkedett miR168
szint térben átfed a vírus
által
elfoglalt területekkel**

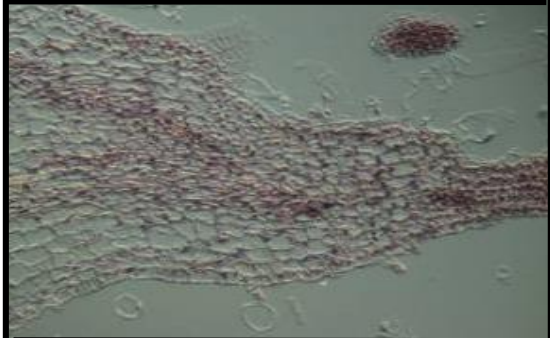
CymRSV



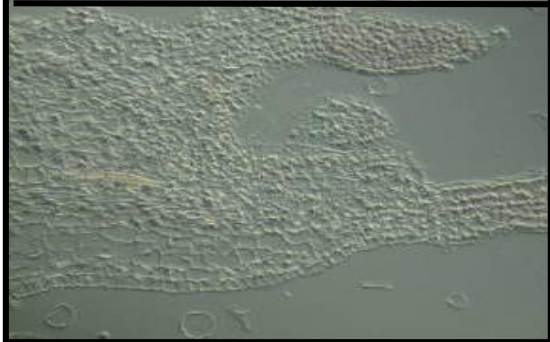
miR168



miR159



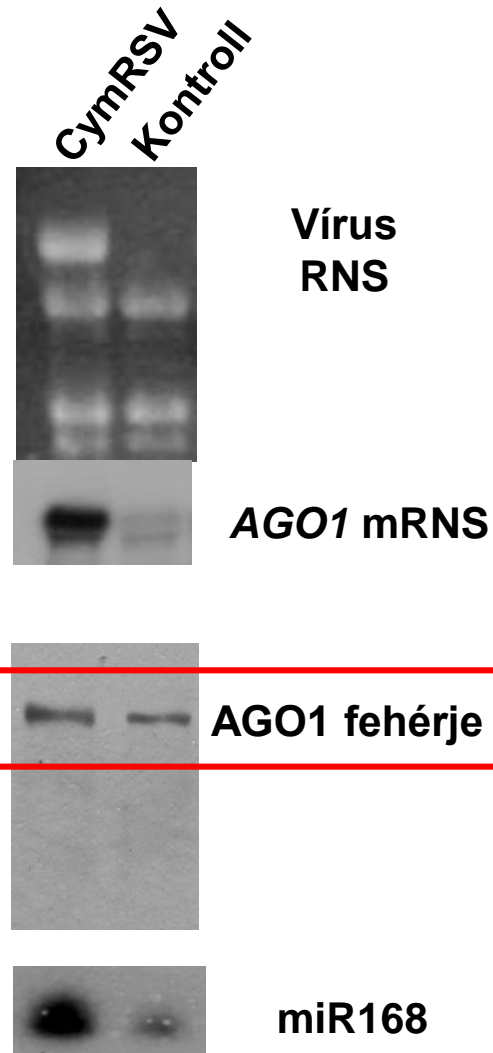
miR449



**Közvetlenül a vírus replikáció
vagy egy vírus eredetű fehérje
indukálja**

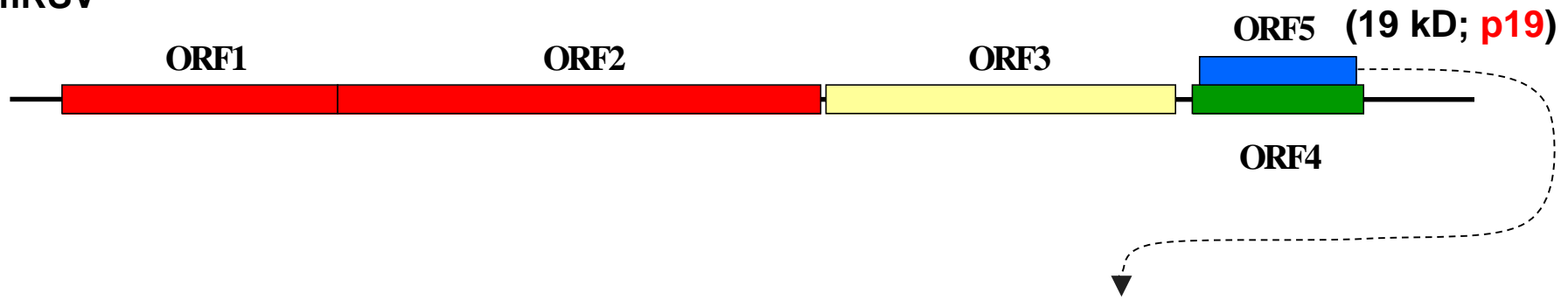
AGO1 fehérje felhalmozódás gátolt a vírusfertőzött növények

N. benthamiana



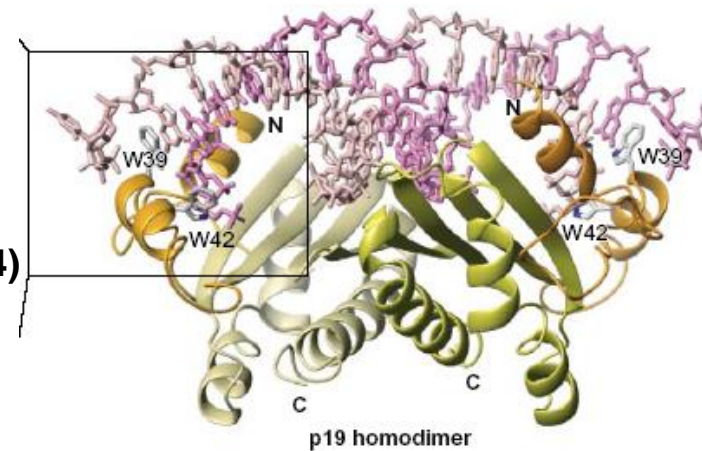
p19 RNS csendesítés gátló fehérje

CymRSV



siRNS kötés

(Silhavy et al., 2002; Lakatos et al., 2004)

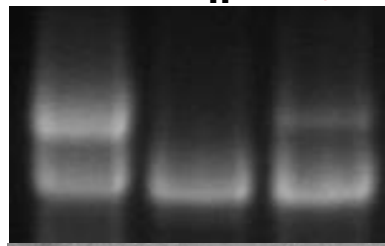
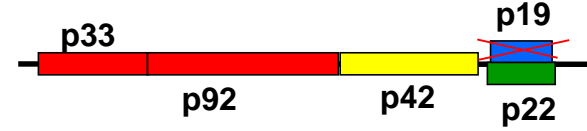


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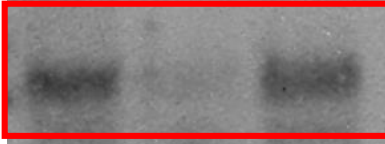
Interferencia a miRNS útvonallal (miR168 indukció) ?

CymRSV Kontro
 || Cym19Stop

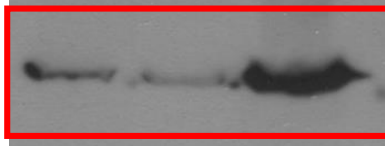
Cym19Stop (Silhavy *et al*, 2002)



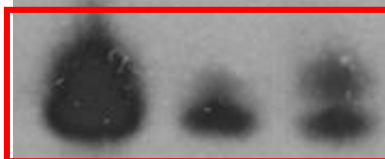
Vírus RNS



AGO1 mRNS

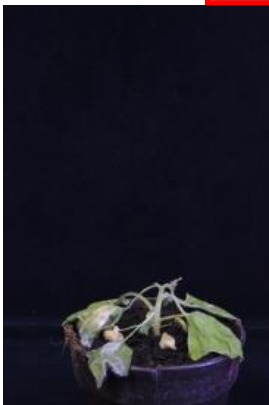


AGO1 fehérje



miR168

N. benthamiana



CymRSV



Kontroll



Cym19Stop



p19 felelős a miR168 indukcióért



Transzlációs gátlás

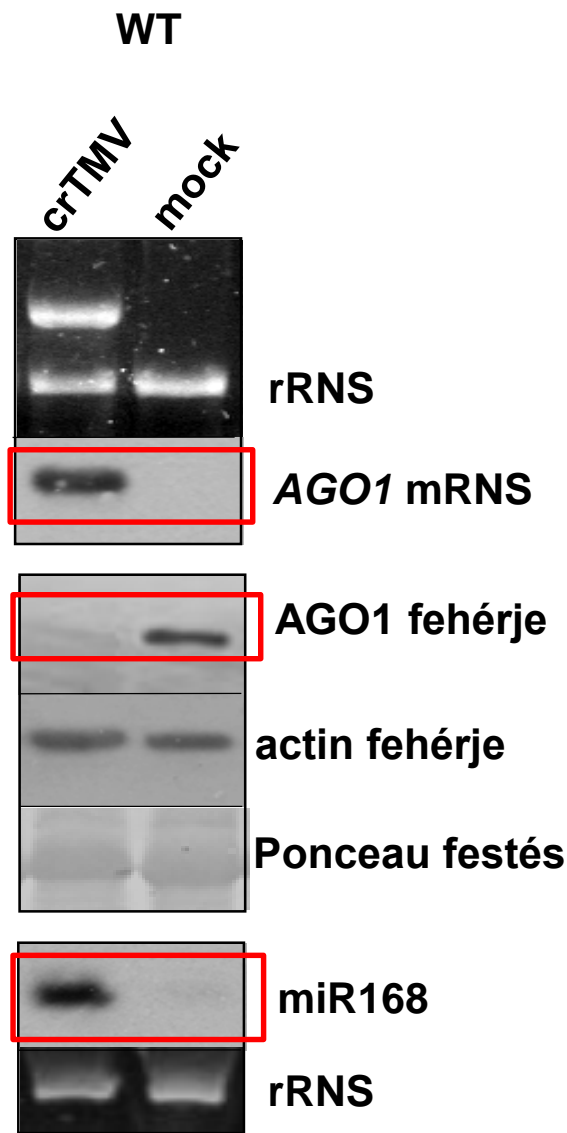
***Arabidopsis thaliana*-ban létezik egy mutáns, amelyben feltételezhető az AGO1 és más miRNS célgének transzlációs gátlásnak az inhibíciója.**

zll-3 (ago10)

(Mallory et al. 2009; Brodersen et al 2008)

A CymRSV nem fertőzi *az A. thaliana*-t.

crTMV fertőzés *A. thaliana*-án a CymRSV fertőzéshez hasonló jelenségeket okoz az AGO1 szabályozás szintjén

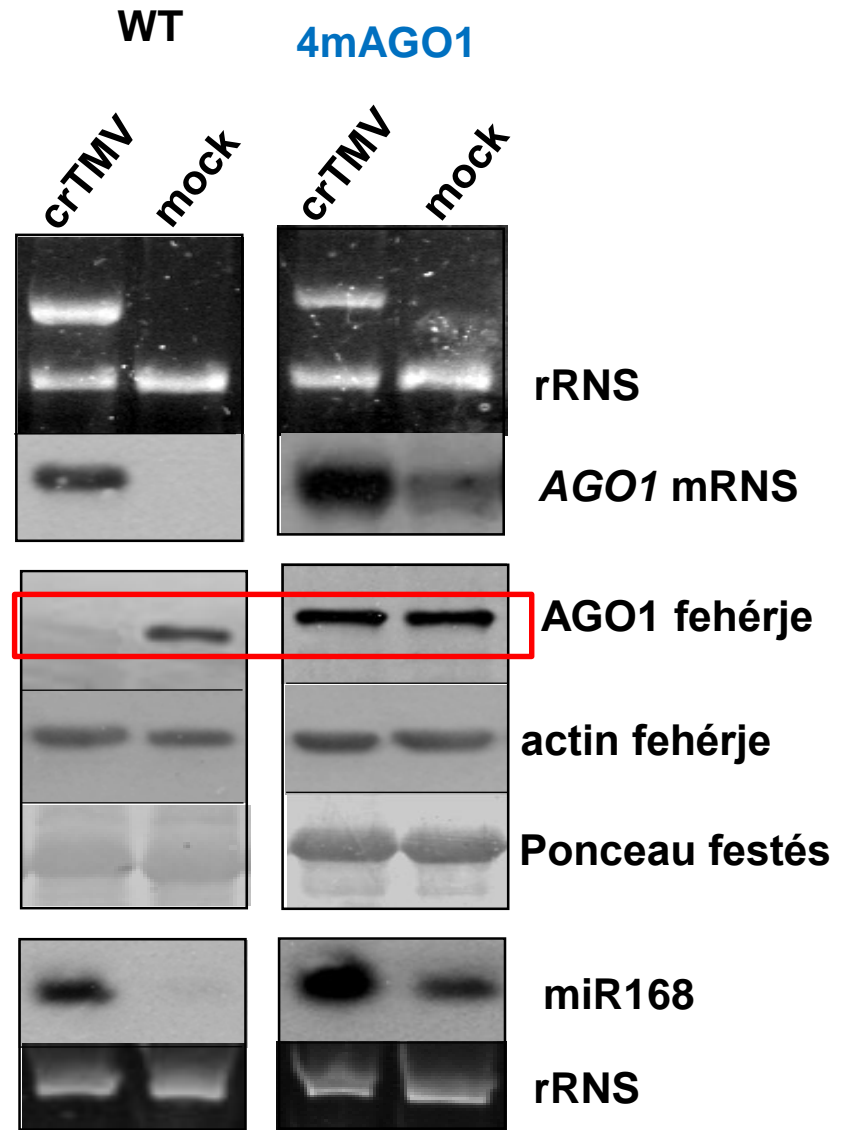


crTMV fertőzött *A. thaliana*-ban az AGO1 gátlás közvetlenül a miR168 aktivitáshoz köthető

4mAGO1

Herve Vaucheret

Olyan AGO1 transzgént tartalmaz, amelynek a miR168 felismerő helye el van rontva



AGO1 gátlás nem működik hatékonyan transzláció gátlás deficiens mutánsokban

