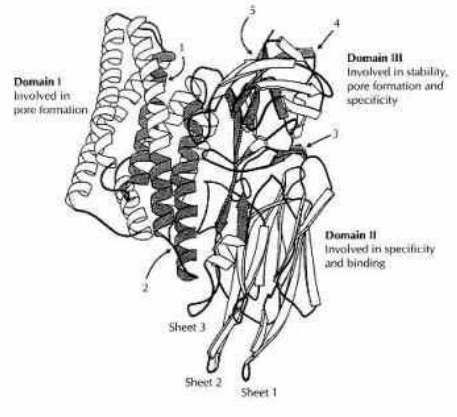
Biosafety considerations of new dsRNA molecules

GM-free Europe Conference 2015
May 6-8
Berlin, Germany

Dr. Sarah Agapito



Current GMOs – Plant transformed to produce new protein

AGCUGGCAA UGU CA

UCGACC GUU ACA GU

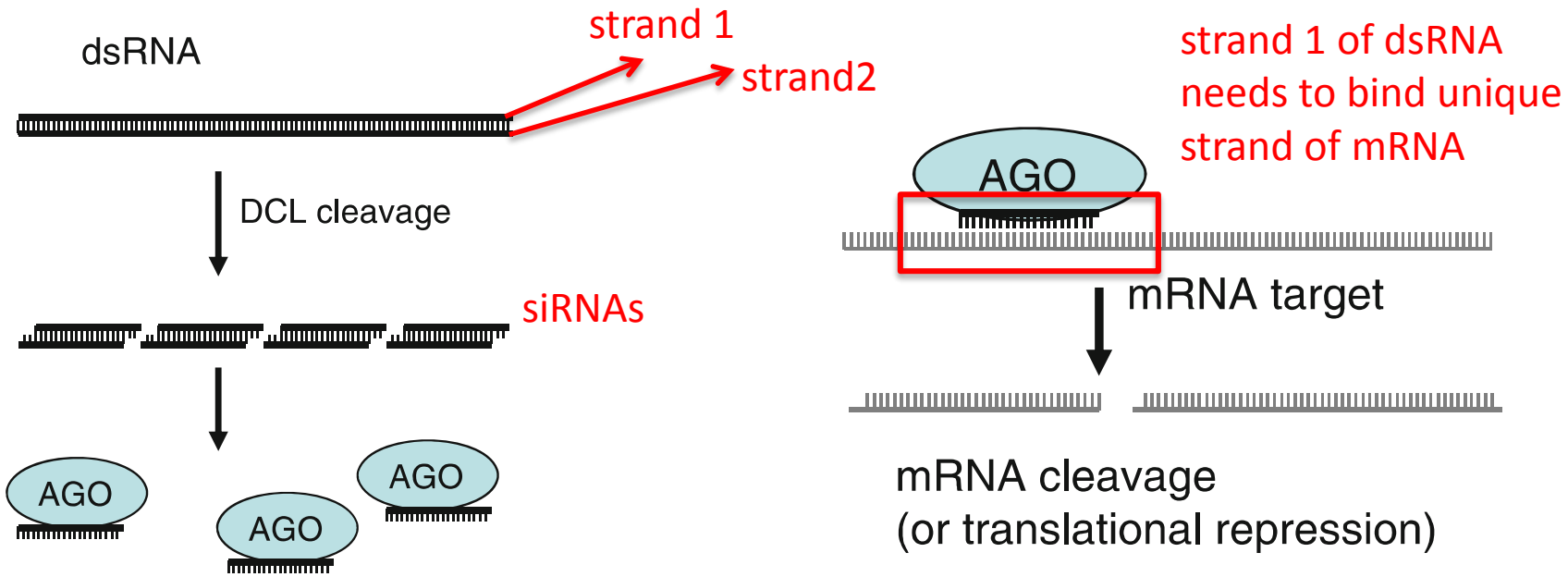
Emerging GMOs – Plant transformed to produce new RNA

Biological processes of dsRNA

- RNA is the genetic information unit responsible for **protein synthesis or inhibition**
- Double-stranded RNA (dsRNA, miRNA, siRNA) is a regulator of the **gene silencing pathway (protein inhibition)**
- This is refered to **RNA interference pathway** and has been discovered as a natural gene control systems in plants, animais and fungi

Biological processes of dsRNA

- **dsRNA inhibits mRNA translation in a sequence homology dependent manner**



genøkoes dsRNA get rapidly degraded?

- **Spraying crude dsRNA** extracts inhibited sugarcane mosaic virus infection (maize dwarf mosaic disease) in field grown maize (Ga

Spray with dsRNA extract - healthy

No spray with dsRNA extract - symptoms

- **Wounded rice leaves soaked** into Agrobacterium suspension with plasmid expressing dsRNA (Andrieu et al., 2012)

Exogenous triggers – promote effective **systemic** supression of endogenos genes and infecting pathogens genes

genøkoes dsRNA get rapidly degraded?

Cell Research (2011) :1-20.

www.nature.com/cr

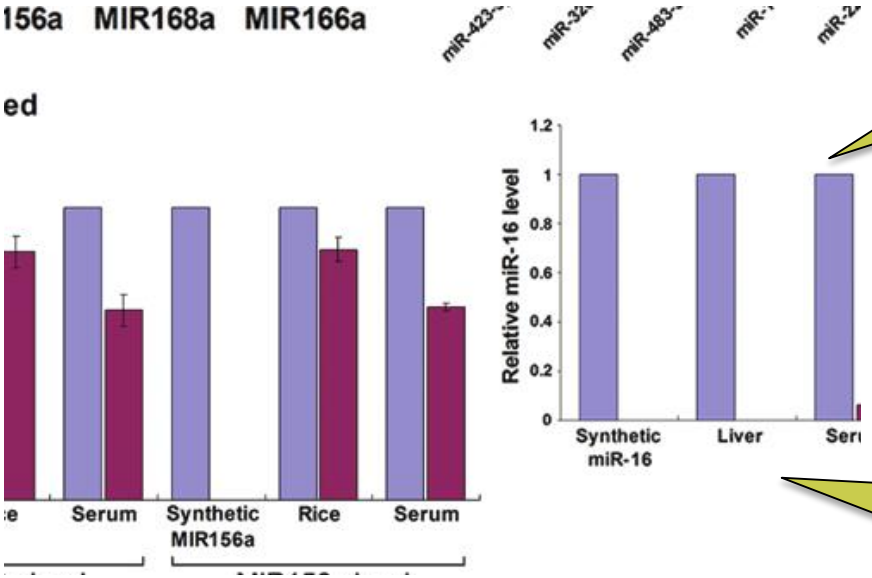
ORIGINAL ARTICLE

Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA

Lin Zhang[1, *], Dongxia Hou[1, *], Xi Chen[1, *], Donghai Li[1, *], Lingyun Zhu[1, 2], Yujing Zhang[1], Jing Li[1], Zhen Bian[1], Xiangying Liang[1], Xing Cai[1], Yuan Yin[1], Cheng Wang[1], Tianfu Zhang[1], Dihan Zhu[1], Dianmu Zhang[1], Jie Xu[1], Qun Chen[1], Yi Ba[3], Jing Liu[1], Qiang Wang[1], Jianqun Chen[1], Jin Wang[1], Meng Wang[1], Qipeng Zhang[1], Junfeng Zhang[1], Ke Zen[1], Chen-Yu Zhang[1]

- **Plant miRNAs** are present in human and **animal sera and organs**
- The exogenous mature **plant miRNAs in food can pass through** mouse **digestive tract** and enter the sera and organs
- **Plant MIR168a** binds to exon 4 of mammalian LDLRAP1 and **decreases LDLRAP1 protein** level in vitro

gen økoes dsRNA get rapidly degraded?



Animal miRNA as control
Absolute levels (RT-qPCR)

- 30 different plant miRNAs in animal serum
- 5% of circulating miRNA is plant
- also found in mouse liver, small intestine, and lung

"Mammalian cells could **selectively pack miRNAs into MVs** in response to different stimuli and then **secrete these MVs into the circulation** of animals or culture medium. Cell-derived MVs could further efficiently deliver miRNAs into the recipient cells where these **exogenous miRNAs regulate the expression of target genes** and the biological functions of recipient cells."

Is dsRNA inherited?

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This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Persistence and transgenerational effect of plant-mediated RNAi in aphids

A.D. Coleman*, R.H.M. Wouters†, S.T. Mugford and S.A. Hogenhout‡

- **exposure of the aphids** to double-stranded RNA (dsRNA)-producing transgenic *Arabidopsis thaliana*
- Target genes were also **down-regulated in nymphs born from mothers exposed to dsRNA**-producing transgenic plants
- RNAi **effect lasted twice as long** (12–14 d) in these nymphs.

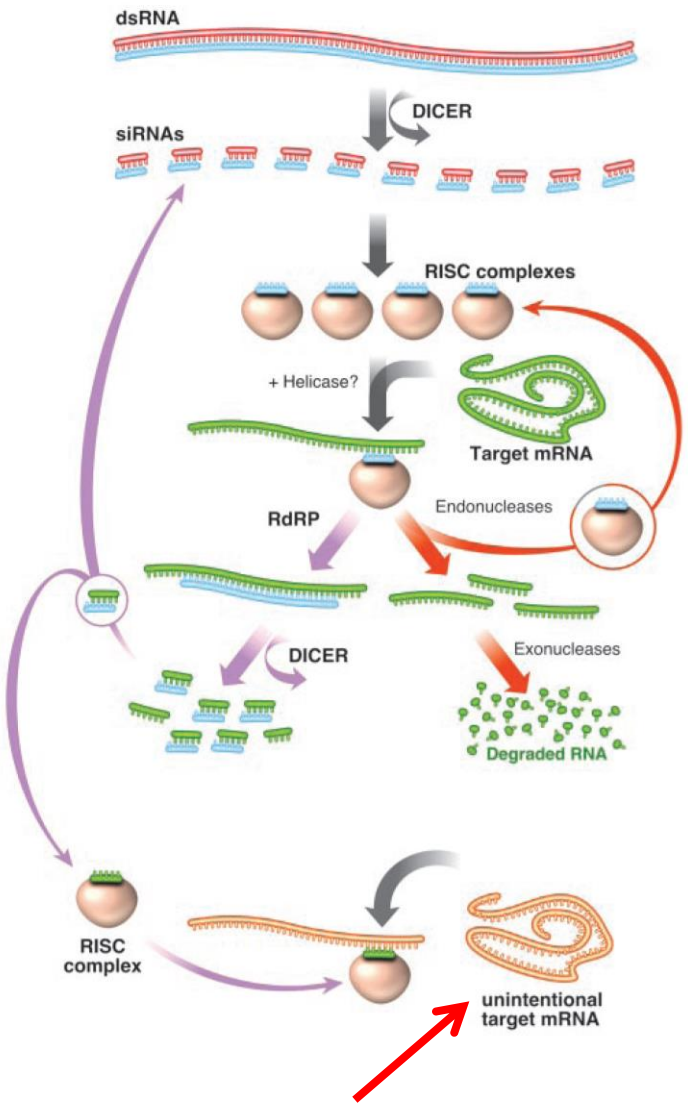
dsRNA amplified off-target effects

dsRNA is specific, but can have unintended effects

RNA dependent RNA polymerases produce new dsRNA **(secondary dsRNA**) from mRNA+miRNA complexes!

Besides the effective target silencing, **a total of 840 genes were regulated** in at least one experiment (**one siRNA sequence**) at the 95% confidence level.

(Semizarov et al., 2003)



dsRNA amplified off-target effects

nature biotechnology

Control of coleopteran insect pests through RNA interference

James A Baum[1], Thierry Bogaert[2], William Clinton[1], Gregory R Heck[1], Pascale Feldmann[2], Oliver Ilagan[1], Scott Johnson[1], Geert Plaetinck[2], Tichafa Munyikwa[1], Michael Pleau[1], Ty Vaughn[1] & James Roberts[1,3]

Amplification (production of other new siRNAs) is unpredictable!
We don't know when it is going to happen and what siRNAs sequences will be produced.

The small amounts of dsRNA required for gene silencing and larval mortality **suggest an amplification pathway** in which ingested dsRNAs are processed to siRNAs, presumably **within insect gut epithelial cells**, which may prime the **synthesis of more abundant secondary siRNAs**, as has been proposed for *C. elegans*.

dsRNA off-target effects

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Early lethality of shRNA-transgenic pigs due to saturation of microRNA pathways*#

Zhen DAI[§1], Rong WU[§1], Yi-cheng ZHAO[1,2], Kan-kan WANG[1], Yong-ye HUANG[1], Xin YANG[1], Zi-cong XIE[1], Chang-chun TU[3], Hong-sheng OUYANG[1], Tie-dong WANG[†‡1], Da-xin PANG[†‡1]

- **shRNA-transgenic pigs for inhibiting** classical swine fever **virus** (CSFV) replication
- great induction of interferon (IFN)-responsive genes in transgenic
- **abnormal expressions of miRNAs** and their processing enzymes are also observed in the livers of shRNA-transgenic pigs, **indicating saturation** of miRNA/shRNA pathways induced by shRNA

- **What is the dose regime for animal/humans feeding dsRNA-expressing GM plants ?**

Scientific evidence around dsRNA safety

- Is dsRNA rapidly degraded in the environment and digestion?
  - ✓ Preferential trasmission or retential (packing and protecting)
  - ✓ Cross-kingdom regulation by microRNA
  - ✓ Persistence and transgenerational effect
- dsRNA can be generally recognized as safe (GRAS)?
  - ✓ Sequenced determined effects
  - ✓ Systemic effects
  - ✓ Amplified effects
  - ✓ Specific biochemical functions – saturation

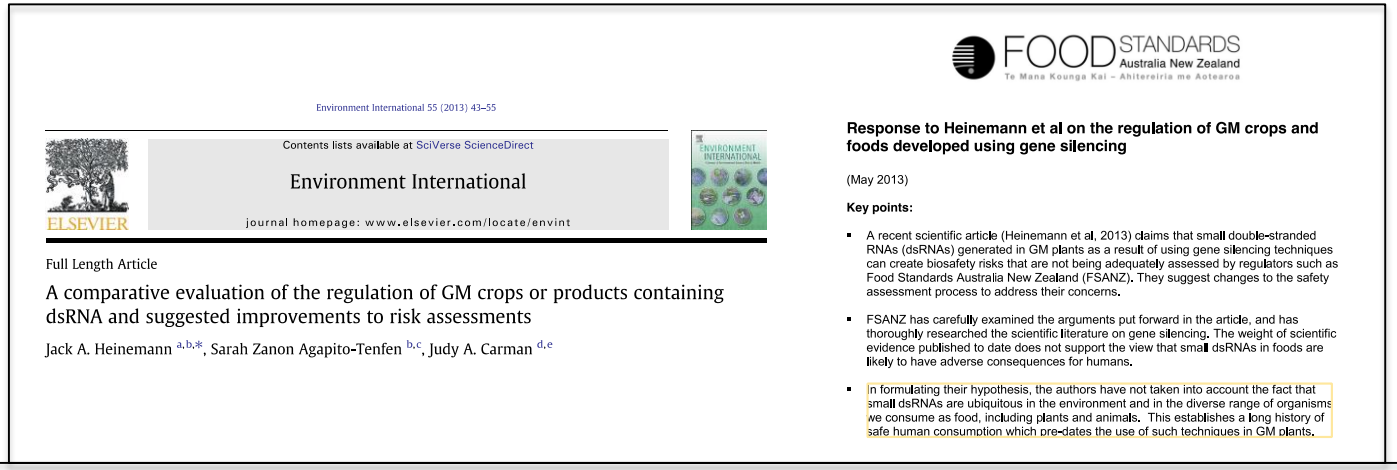
✓ **Regulators should be testing dsRNA for thir safety!**

Challenges

Spray dsRNA – air containment?

Summary

- **Genetic engineering process can create both primary and secondary dsRNAs**
- **These dsRNAs may be amplified and persist in recipient organisms**
- **Since active dsRNAs are small, there may be many unintended targets**
- **Emerging developments could see pesticide sprays and direct-to-consumer products intended to transfer dsRNAs**
- **dsRNA spray could be considered *in-field* transformation technique**



Environment International 55 (2013) 43–55

Contents lists available at SciVerse ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full Length Article

A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments

Jack A. Heinemann [a,b,*], Sarah Zanon Agapito-Tenfen [b,c], Judy A. Carman [d,e]

FOOD STANDARDS Australia New Zealand

Te Mana Kounga Kai – Ahitereiria me Aotearoa

Response to Heinemann et al on the regulation of GM crops and foods developed using gene silencing

(May 2013)

Key points:

- A recent scientific article (Heinemann et al, 2013) claims that small double-stranded RNAs (dsRNAs) generated in GM plants as a result of using gene silencing techniques can create biosafety risks that are not being adequately assessed by regulators such as Food Standards Australia New Zealand (FSANZ). They suggest changes to the safety assessment process to address their concerns.
- FSANZ has carefully examined the arguments put forward in the article, and has thoroughly researched the scientific literature on gene silencing. The weight of scientific evidence published to date does not support the view that small dsRNAs in foods are likely to have adverse consequences for humans.
- In formulating their hypothesis, the authors have not taken into account the fact that small dsRNAs are ubiquitous in the environment and in the diverse range of organisms we consume as food, including plants and animals. This establishes a long history of safe human consumption which pre-dates the use of such techniques in GM plants.

Regulatory Toxicology and Pharmacology 71 (2015) 8–23

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

A 28-day oral toxicity evaluation of small interfering RNAs and a long double-stranded RNA targeting vacuolar ATPase in mice

Jay S. Petrick*, William M. Moore, William F. Heydens, Michael S. Koch, James H. Sherman, Shawna L. Lemke

Regulatory Toxicology and Pharmacology 71 (2015) 599–600

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Regulatory Toxicology and Pharmacology

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Letter to the Editor

Response to "A 28-day oral toxicity evaluation of small interfering RNAs and a long double-stranded RNA targeting vacuolar ATPase in mice."

Regulatory Toxicology and Pharmacology 71 (2015) 597–598

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Letter to the Editor

Authors' Response to Letter to the Editor by Heinemann et al. "Response to 'A 28-day oral toxicity evaluation of small interfering RNAs and a long double-stranded RNA targeting vacuolar ATPase in mice.'" 2015

particles ranging in size from 90 to 100 μm (Goldstein et al., 2004), in contrast with respirable particles that are less than 10 μm. Thus pollen is deposited in and cleared from the upper respiratory tract, resulting in secondary oral exposure rather than pulmonary exposure. Therefore, when conducting hazard identifi-

Genome-wide microRNA expression profiling reveals pleiotropy in transgenic crops. Sarah Zanon Agapito-Tenfen[ab*], Vinicius Vilperte[b*], Terje Ingemar Traavik[a], Rubens Onofre Nodari[b] (under submission). – EFSA Conference Oct