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Early warning on food safety issues: how regulators got it wrong on dsRNA

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Abstract

Changing the nature, kind and quantity of particular regulatory-RNA molecules through genetic engineering can create unique risks. For some GMOs, this outcome is intended but for many others it is not. To characterise, assess and then mitigate potential adverse effects arising from changes to RNA will require a different approach to food and environment risk assessments of GMOs. I will discuss the advice offered to a government regulator during official risk evaluations of GM plants for use as human food, how the regulator dismissed those risks a priori, and what that experience teaches us about the risk assessment framework.

Introduction

All commercialised GM plants at this time are created through in vitro DNA modification. However, not all of them are created with the intention to produce a new protein. A growing minority are designed to change their RNA content (Table 1). The reason for this is the finding that RNA, specifically double-stranded RNA (dsRNA), is an important regulator of gene expression (Appendix 1 of Heinemann, 2009). In the near future, GM products may arise from only in vitro RNA modification (Heinemann, 2009).

RNA is an intermediate molecule used in the cellular reactions of translation to synthesise proteins. The most familiar form of RNA is mRNA, the single-stranded messenger. However, it is only in the last 10-15 years that small dsRNA molecules have become known for their role in regulating gene expression (Hutvágner and Simard, 2008).

dsRNAs are variously called siRNA (short-inhibitory RNA), miRNA (microRNA), shRNA (short-hairpin RNA) and so on and are foundation substrates in biochemical pathways that cause RNAi (RNA interference), PTGS (co-suppression, post-transcriptional gene silencing) and TGS (transcriptional gene silencing). In short, RNAi, PTGS and TGS are caused by gene silencing: disrupting the connection between genes and the production of the proteins specified by genes¹.

dsRNAs form when both strands of a DNA molecule are transcribed to synthesise complementary RNA molecules (which then bind together in the same way as strands of DNA), or when stretches of intra-molecular complementarity create stem-loop structures (Figure 1). A long dsRNA molecule (e.g., pre-mature miRNA) is processed into a shorter dsRNA (e.g., miRNA) and then one strand is retained – the guide strand – to direct protein complexes to target mRNA molecules and prevent their translation (cytoplasmic pathways), or to target and chemically modify DNA sequences by addition of methyl groups and cause modification of DNA-associated histone proteins (nuclear pathway). The nuclear pathway is known to inhibit transcription and to seed heterochromatin formation (Ahlenstiel et al., 2012, Grewal and Elgin, 2007, Reyes-Turcu and Grewal, 2012, Zhang and Zhu, 2012).

¹ For an excellent animation, see <http://www.nature.com/nrg/multimedia/rnai/animation/index.html>

Table 1. Various GM crops with intended RNA changes in the food approval pipeline

Product	Status	Ref/Application Code
Flavr Savr Tomato	withdrawn from market	(Sanders and Hiatt, 2005)
High oleic acid soybean lines G94-1, G94-19 and G168 ²	FSANZ ¹ approved (2000)	A387
New Leaf and New Leaf Plus Potatoes ³	FSANZ approved (2001)	A383 and A384
High oleic acid soybean line DP-305423-1	FSANZ approved (2010)	A1018
Herbicide-tolerant, high oleic acid soybean line MON87705	FSANZ approved (2011)	A1049
Golden mosaic virus resistant pinto bean	Brazil approved (2011)	(Tollefson, 2011)
papaya ringspot virus resistant papaya	USA (1996), Canada (2003) and Japan (2011)	USDA ⁴ GMO Compass ⁵

¹ Food Standards Australia/New Zealand (FSANZ)

<http://www.foodstandards.gov.au/consumerinformation/gmfoods/gmcurrentapplication1030.cfm>

² “Withdrawn from [FSANZ] Standard 1.5.2 in 2011 because never commercialised.”

³ The way the virus protein gene used as a transgene causes resistance to the potato viruses (Y and PLRV) was unknown at time of approval. However, it is well known now that gene duplications (which occur when the virus infects the GM plant) cause silencing of both copies of the gene through RNAi.

⁴ http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Japan%20approved%20GM%20papaya_Tokyo_Japan_12-19-2011.pdf

⁵ <http://www.gmo-compass.org/eng/database/plants/59.papaya.html>

Once a silencing effect is initiated, the effect may be inherited. The biochemistry of this process varies depending on organism and remains an area of active research with many unknown aspects. Nevertheless, it is known for example that human cells can maintain the modifications necessary for TGS, creating actual or potential epigenetic inheritance within tissues and organisms (Hawkins et al., 2009).

Unintended gene silencing is a common outcome of the genetic engineering process. Indeed, most cells initially engineered using in vitro nucleic acid techniques ultimately “silence” the gene inserted because of the engineering-associated production of dsRNA (Denli and Hannon, 2003, Weld et al., 2001). The new RNA sequence may be created when the DNA strand not normally used as a co-factor for transcription is used as such (perhaps because the insert had a cryptic promoter activity or inserted near a promoter). The resulting single-stranded RNA may bind to the target mRNA to create regions of linear dsRNA that can be processed into siRNA (Figure 1). Another possibility is that the insert contributes to the formation of a stem-loop, from which the “stem” may be processed into an miRNA-like molecule (Figure 1).

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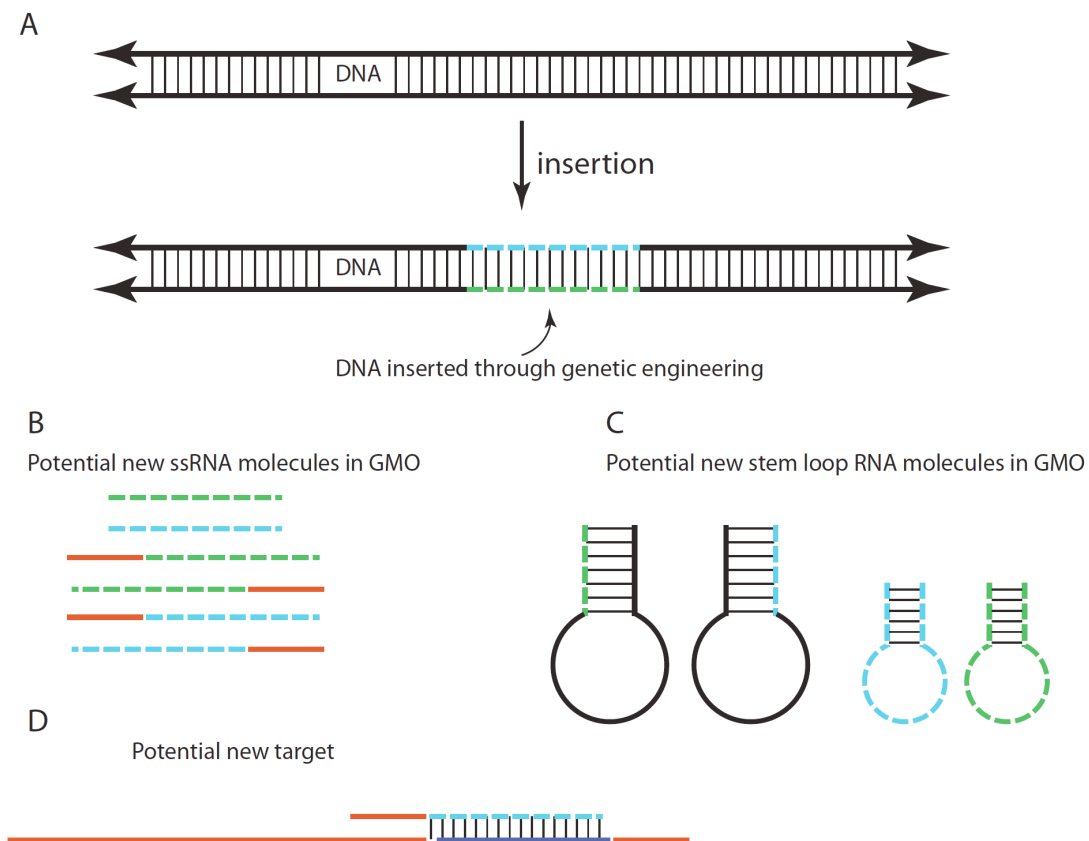


Figure 1: source of new dsRNA molecules from genetic engineering.

(A) Regardless of the source of the DNA inserted (dashed blue and green lines in the black double stranded DNA molecule) into a genome by genetic engineering, it creates new sequences. The DNA used will create new sequences because it will be bordered (boundary between dashed and solid lines) by different sequences than in the source genome by the engineering process, or may be sourced from a genome that has no or few sequence matches. (B) Transcription will produce new RNA molecules (red and dashed blue and green lines) that might be able to form dsRNA because of complementarity or (C) because of internal base-pairing causing stem-loop structures to form (base-pairing illustrated with thin black connecting lines). (D) This may lead to intended and off-target (red line with purple target section) gene silencing in the GMO or in organisms that eat the GMO.

Summary

dsRNAs are remarkably stable in the environment. Insects and worms that feed on plants that make dsRNA can take in the dsRNA through their digestive system, where it remains intact (Gordon and Waterhouse, 2007, Mao et al., 2007). Worms can absorb dsRNA through their skin when dsRNA is suspended in liquid (Cogoni and Macino, 2000, Tabara et al., 1998). Once taken up, the dsRNA can circulate throughout the body and alter gene expression in the animal (Mello and Conte Jr., 2004). In some cases, the

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dsRNA taken up is further amplified or causes a secondary reaction that leads to more and different dsRNAs ("secondary" dsRNAs) with unpredictable targets (Baum et al., 2007, Gordon and Waterhouse, 2007).

New dsRNA molecules can be made as a side effect of genetic engineering (or existing dsRNA molecules can be made in higher or lower quantities), and some GMOs were created for the purposes of generating new dsRNA molecules. Some dsRNA molecules can have profound physiological effects on the organism that makes them. Some dsRNA molecules can be transmitted through food or other means to other organisms and can have effects on these organisms that are not yet understood. "A daunting outcome is raised, that each [dsRNA] formulation might have its own risks" (p. 514 Aronin, 2006).

There are no validated procedures for excluding either exposure pathways or potential adverse effects of particular dsRNA molecules that may be produced as a result of genetic engineering, whether intended or otherwise. Therefore, for the foreseeable future, all GMOs intended for release or food should be submitted to a battery of testing for unknown dsRNAs and unintended effects of dsRNAs. The testing should provide empirical evidence capable of providing confidence for claims of the absence of any unintended dsRNAs or of an unintended effect.

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