



Food and Agriculture Organization
of the United Nations

What is RNAi and why it is relevant to our Food?

A seminar by Nobel Laureate, Dr Andrew Fire

8 May 2015

Food and Agricultural Organization of the United Nations
Rome, Italy

1 Acronyms

DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
RISC	RNA-induced silencing complex
RNAi	RNA interference
mRNA	messenger RNA
siRNA	small interfering RNA
dsRNA	double stranded RNA

2 Introduction

2.1 Background

At 8 May Nobel laureate Dr Andrew Fire from Stanford University visited FAO to deliver a seminar entitled 'RNAi and why it is relevant to our food'. The seminar specifically aimed to increase the understanding of the audience on the scientific concepts that underlie RNA interference (RNAi) and how these can be relevant to the food and agricultural domain.

2.2 Seminar agenda

The seminar followed the agenda below:

Time	Item	Venue
10.00	Welcome by Daniel Gustafson, DDO Opening remarks Ren Wang, ADG/AG	Iran Room (B116)
	Seminar by Dr Andrew Fire: "What is RNA interference (RNAi) and how this can be relevant to our food?"	
	Q and As	
	Discussions	
12.30	Closing	

Due to copyright issues no slides or recordings of this event will be made available.

2.3 Biography of Dr Andrew Fire

Dr Andrew Fire is a professor of pathology and of genetics at the Stanford University School of Medicine. He was awarded the 2006 Nobel Prize for Physiology or Medicine, along with Dr Craig C. Mello, for their contributions in the discovery of RNAi. Dr Fire and Dr Mello, along with other colleagues reported that tiny snippets of double-stranded RNA (dsRNA) effectively shut down specific genes, removing messenger RNA (mRNA) with sequences matching the dsRNA. As a result, the mRNA cannot be translated into protein. Dr Fire and Dr Mello found that dsRNA was much more effective in gene silencing than the previously described method of RNA interference with single-stranded RNA. Because only small numbers of dsRNA molecules were required for the observed effect, Dr Fire and Dr Mello proposed that a catalytic process was involved. This hypothesis was confirmed by subsequent research. Dr Fire was also a recipient of the Meyenburg Prize (2002), NAS Award in Molecular Biology (2003), Wiley Prize (2003) and Massry Prize (2005) prior to be awarded the 2006 Nobel Prize for Physiology or Medicine.

3 An FAO seminar: What is RNAi and why it is relevant to our food?

3.1 Welcome remarks by Mr Daniel Gustafson, DDO, FAO

Daniel Gustafson, Deputy Director General of FAO, welcomed Dr Fire and the audience by stating that he was delighted to welcome such a highly recognized scientist to FAO. He stressed that science and technology is at the forefront of FAO's activities and that much work pursued by FAO has a scientific component. It is of importance to address questions on how science can be used, brought across to the general public and incorporated into policies. He also recommended participants to read the Nobel Lecture¹ that Dr Fire delivered upon receiving the Nobel price as it was very insightful for non-experts.

3.2 Presentation by Dr Fire

3.2.1 *The discovery of RNA interference*

In the first part of his presentation Dr Fire spoke about how RNAi was discovered. Starting from the first unexpected observations that exogenous RNA fragments can inhibit the expression of complementary genes in Fungi, Plants, and *Caenorhabditis elegans*, Dr Fire elaborated on how the underlying molecular processes of this process were subsequently further unraveled. Experiments in which the RNA fragments were separated by gel showed that the effective molecules were double stranded. Furthermore, the silencing of genes was achieved by the degradation of RNA fragments produced by the targeted organism. Also when the fragments were injected in other places than the gut an effect was observed. When *C. elegans* was fed with bacteria that were engineered to produce dsRNA, targeted gene silencing could be achieved.

After these observations the question arose whether the observed effect was caused by the physio-chemical properties of dsRNA or whether an intrinsic cellular process was involved. If the latter was the case this would imply that certain mutant worms might be insensitive to dsRNA. By inducing random genetic modifications through mutagenesis, worms could be produced that were resistant to any major effects of dsRNA. The genetic sequence of the worms showed which genes were involved in the silencing process.

Looking beyond *C. elegans* dsRNA effects also occur in other organisms, including plants and invertebrates. Mammalian organisms are seen to shut off a large part of the cellular mechanism: vertebrates can get flu-like symptoms when injected with dsRNA and this made initial investigations of more specific mechanisms a challenge. In plant systems, it was shown that RNAi acts as a viral defense mechanism, once a virus replicates it transiently consists of a dsRNA sequence that is recognized by the plant cell and invokes the response.

Biochemistry experiments were done to identify involved proteins and to characterize the process. Once the dsRNA fragment enters the cell it is recognized by a complex that binds the fragment and cut it into smaller pieces. These smaller fragments are loaded on a second complex, the RNA-Induced-Silencing Complex (RISC) that searches the cell for the same identical sequence to degrade it. The degradation of the specific RNA sequence results into the silencing of the targeted gene. The efficiency of the silencing process in a subset of organisms (plants, worms, fungi) can be explained by

¹ Link: http://www.nobelprize.org/nobel_prizes/medicine/laureates/2006/fire_lecture.pdf

the amplification of the signal by RNA-directed RNA polymerase, an enzyme that multiplies the population of RNA fragments.

In mammalian cells the use of small interfering RNA (siRNA) fragments can trigger a cellular response and result in a ceased gene expression. The delivery of these fragments to mammalian cells remains challenging and hence impedes the therapeutic applications of RNAi. Various chemical delivery strategies have been explored and these seem to be most effective in liver and eye tissue. The United States' Food and Drug Administration so far did not approve any pharmaceuticals or treatments that rely on RNAi.

3.2.2 Applications of RNAi in agriculture

In the second part of his presentation Dr Fire specifically addressed the potential applications of RNAi in the food and agricultural sector.

Dr Fire specifically spoke about possible strategies relying on RNAi to replace fumigants to combat nematodes in agriculture. In the past methyl bromide was used to sterilize the soil before planting to prevent infection. However, since this substance is very toxic this practice bared hazards for agricultural workers and adjacent communities. Methyl bromide also has been implicated in harming the ozone layer. The latter gave rise a ban on Methyl Bromide and hence to the question whether more natural strategies might be used as alternative in the future. In this context RNAi can provide an effective alternative: by developing plants that produce specific RNA sequences that silence genes essential for nematode survival, the pest can be effectively removed from the field. Dr Fire showed an example of carrot that was stunted in its growth due to nematode infection as an example of a case where dsRNA targeting nematode genes would certainly be a useful approach. Possible beneficial applications in the agricultural domain can include the development of plants with changed intrinsic properties or an altered interaction with their environment. For example plants could be developed that are resistant to environmental stress (e.g. drought), have an increased post-harvest stability, improved taste and culinary properties or have decreased levels of toxins and/or allergens. When considering interaction with the environment, a plant can be developed that shuts off plant genes involved in pathogen sensitivity or that can transfer dsRNA produced by the plant to pathogen once it is eaten. Effectiveness of RNAi in replacing conventional herbicide approaches is also depends on how it is delivered in the field; so-far studies seem to be encouraging.

Possible negative consequences to consumers can be a drawback for using RNAi. A relevant consideration in this perspective is whether people exist that show a strong negative response when exposed to ingested dsRNA. Much of our commercial produce contains double stranded RNA, and for at least the vast majority people consuming dsRNA there is no negative response. Another consideration are the characteristics of the modification: if a plant is developed that harbors genes encoding substances that exert negative properties, this might affect the safety. Finally, unintended effects of the modification can result in safety considerations. For example when unintended plant genes are shut off or when the dsRNA silences homologous genes in other non-target organisms. Dr Fire concluded his presentation by advocating an effective and rapid regulatory infrastructure that protects human health and the environment and enables beneficial advances that could in particular minimize the use of highly toxic chemical pesticides. This might allow the technology to make a contribution to improve the safety of agriculture and to provide beneficial tools for future agriculture development.

3.3 Questions and discussions

Q1: The current regulatory framework on biotechnology has many safeguards but that the systems must not inhibit the good use of the technology. Is there a way how a rough classification can be made about what products can be reasonably considered safe and of which we have to be more careful?

Dr Fire: In my perspective the scientific community should regulate itself but should not be solely responsible for regulating itself. A regulatory infrastructure should be fast enough and enabling for beneficial applications. This structure should focus on monitoring health effects of agricultural workers and communities as these are the most directly exposed.

Q2: RNAi technology is out now for almost a decade. What is the current status of practically applying the technology?

Dr Fire: RNAi is an effective technology for the modification of plants by shutting off genes. For example, the American company Simplot developed a potato variety that has improved storage characteristics and lower levels of acrylamide.

Q3: DsRNA viruses are widely transmitted animal pathogens. What is the specific trick by which they can enter mammalian cells? And can this provide a potential delivery strategy for the therapeutic use of RNAi?

Dr Fire: Studying dsRNA viruses can be useful since they circumvent cellular response by blocking the machinery that transports RNA fragments throughout an organism. To date many research efforts are made on how viral proteins can be used in the delivery of dsRNA to mammalian cells for therapeutic purposes.

Q4: How can the public be engaged in the regulatory structure for this technology?

Dr Fire: In my opinion the scientific community should not be solely responsible for regulating itself. However, scientists have the responsibility to communicate to the public about the certainties and uncertainties relating to the use of technology. The discussion on biotechnology should be held in a context that addresses the alternatives by prioritizing risks of different intervention strategies. Human interventions in agriculture are needed and it is of importance that people better understand the basics of agriculture and plant sciences. Some people might have intrinsic resistance to biotechnology, whereas others acknowledge the increasing pressure to overcome drought and reduce pesticide use.

Q5: RNAi can have potentially lower toxins and anti-nutrients in animal feed. Why is this potential not yet realized?

Dr Fire: This is an interesting biological question. Biochemistry is needed to identify targets and develop products that actually work.

Q6: When considering the importance of responsible governance of this technology, how do you see the interaction between scientists and international organizations?

Dr Fire: Collaboration between scientists and international organizations like FAO on all different levels is extremely important. It is not only very important to have renowned

scientists engaging in the work international organizations, but also to give young scientists the opportunity to see how these organizations work.

Q7: By developing sterile insect technology a big constraint is how that can be applied in accordance with good agricultural practices. What is your perspective on this?

Dr Fire: Good practices are essential but that it has to be noted that there will always be a chance that accidents will happen. Also the chance exists that technologies can be abused and therefore more effort should be made on how develop technologies is such a way that abuse can be prevented.