mRNA Interference: A Possible Cure for Bluetongue?

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PASS WITH MERIT

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In this paper, I am going to discuss the use of RNA interference, describing the process which resulted in its discovery and current developments in RNAi research. The main focus will be on how RNAi could be used to treat Bluetongue disease, and how some of the problems researchers are facing could be overcome, again using Bluetongue as an example. It will also briefly cover some of the ethical issues associated with the treatment.

RNA is responsible for carrying protein synthesis instructions from DNA in the nucleus to the ribosomes, which are the sites for protein synthesis. The DNA unwinds through the breaking of hydrogen bonds to expose a sequence coding of bases. RNA nucleotides then form a messenger RNA (mRNA) molecule through complimentary base pairing with the gene. The mRNA then separates from the DNA and leaves through pores in the nuclear envelope into the cytoplasm where it attaches to the ribosomes.

It is possible to stop this process so that the mRNA never reaches the ribosome, which in effect "turns the gene off".

A method of preventing transcription can be seen to naturally occur in simple eukaryotic organisms, for example plants and invertebrates, when they are invaded by a viral form which contains RNA. The virus will have a "specific genetic fingerprint" and many viral forms contain double-stranded RNA (dsRNA), which is identified by the organism. The invader enters a pathway known as RNA interference pathway. The long molecule is cut into sections by an enzyme called DICER, and this in turn activates small interfering RNA (siRNA) molecules to assemble into RNA-induced silencing complexes (RISC). These complexes open up the short dsRNA and copy the genetic fingerprint of the viral form. This means the cells are able to destroy anything with a matching RNA fingerprint. (Figure 1)

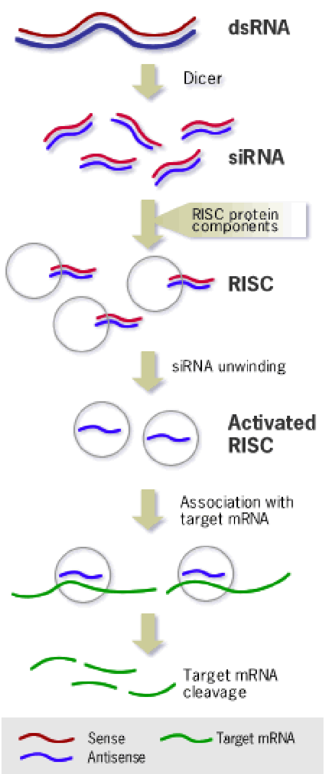

Figure 1

In more complex organisms this defence system is not used; instead the long dsRNA chains provoke a complicated inflammatory response which leads to the shutdown of all protein synthesis in the infected cell. However the RNAi pathway is present in these organisms, and can recognise and destroy foreign RNA and indeed its own mRNA. It has been found that the normal antiviral system can be bypassed by introducing siRNA, which triggers the interference response, without triggering the inflammatory response.

This leaves us with a wealth of possibilities, such as possible cures for any disease, including cancer, and the control of gene expression, as well as allowing greater research into gene function.

The principles of gene expression were identified by French scientists François Jacob and Jacques Monod, and it is these principles which form the basis for gene technology. In 1990 the work of plant biologists experimented with added genes to increase colour intensity in petunias; their result was at the time inexplicable as the plants lost instead of gained colour, and the mystery was only solved by a discovery by Andrew Fire and Craig Mello in 1998. Their experiments with nematode worms led to discover that double stranded RNA can act as a trigger for gene silencing. They were injecting the worms with mRNA (a "sense" sequence) and "antisense" RNA separately, to no effect, but found when they injected the two together they found significant changes that indicated a halt in protein synthesis. Eventually they realised the sense and antisense RNA strands were binding to form dsRNA, and after further experimentation they discovered how this in turn silenced the genes. The mystery of the colourless petunias could now be explained. The men received the Nobel Prize in 2006 for their work.

At the moment the use of mRNAi is still at a research level, though it is gradually filtering into medicine and veterinary medicine as a novel treatment for many diseases such as cancer and the College for Veterinary Medicines, University of Georgia, are currently developing new antiviral drugs that use this method of gene silencing, with the claim that "Our long-term goal is to develop novel in vivo cell targeting and delivery approaches for siRNA candidates to be used therapeutically across a range of virus strains" . However there are many problems with RNAi, such as the question of how to deliver the siRNA, and how to cope with the constant mutations of viruses.

Bluetongue is a disease which affects mainly sheep, cattle and other ruminants and is caused by a virus, genus *Orbivirus*, family *Reovirades.* There are currently 24 serotypes of the disease, many of which is can be found in Europe (Figure 2). Animals mainly show signs on the mucous linings and the coronary band, just

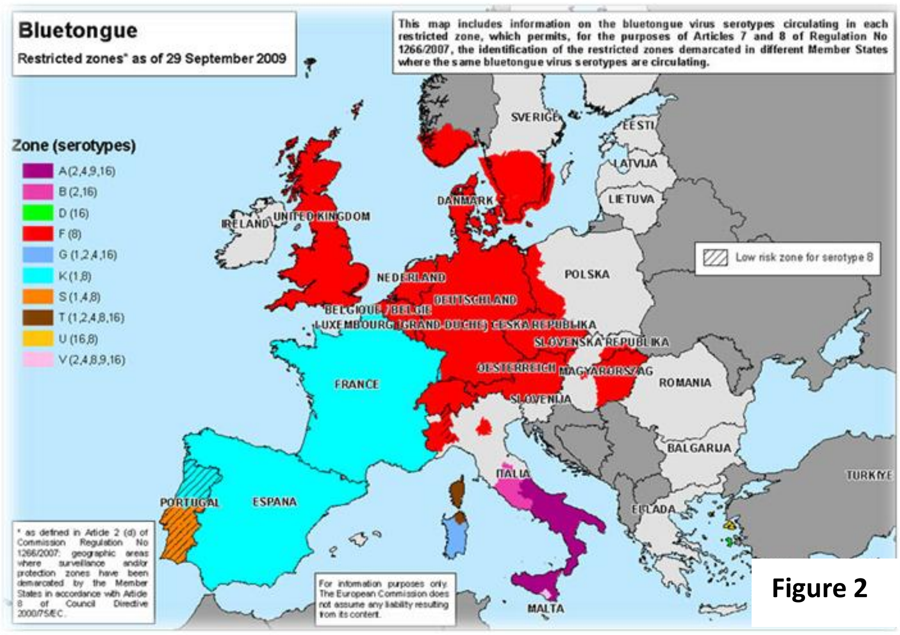

Figure 2

above the hoof. It is transmitted by a vector (a few species of midges) but cannot be transmitted directly between animals, although there is a possibility of mechanical transmission. It contains an RNA double strand, and is therefore a prime example of a disease that could be treated by RNAi.

It does not affect humans, but can cause great economic losses for farmers. It affects sheep more severely than cattle, with a mortality rate of up to 70% in flocks. The disease can also cause infertility, chronic problems such as lameness. In 2007, Robert Law, a farmer in Hertfordshire, lost £8000 after nearly half his rams were found to be infertile due to bluetongue. Milk yields in dairy cattle can drop by 40%.

The first recorded case of the disease in Great Britain was in September, 2007, and the increase of the disease can be blamed on numerous factors including increase of travel, especially between EU countries, where the disease is far more prevalent, and global warming increasing the number of midges. There is a vaccination against the disease, but no cure, so those animals infected with the disease are often condemned to slaughter, resulting in both financial and emotional losses for farmers. The vaccination also requires an annual booster for all animals.

So how would RNAi be used to treat bluetongue? Simply an infected animal would have siRNA inserted in order to override the inflammatory response so that the virus was sent down the RNAi pathway resulting in the destruction of the virus. However it is not that simple; as stated above there are numerous problems with delivering the siRNA, keeping the levels of siRNA present if the animal is not cured instantly and dealing with viral mutations, for example there are currently 24 known serotypes of bluetongue. There are also financial problems to consider, and the ethical questions that accompany any sort of genetic treatment.

One possibility for keeping constant levels of the treatment over a long period of time may be an implant that releases measured amounts of the siRNA after a certain period of time. This might possibly solve the delivery problems, though at the moment the main problem with this is the toxicity of the delivery agents. Implants are often used in hormone treatment, for example growth promoting implants in cattle; this allows us to look at the possible advantages and disadvantages of using this method.

Growth hormone implants do not require a withdrawal period, however this is difficult to compare with the possible effects of siRNA implants, as it is yet unknown if it is possible for siRNA to pass down the food chain; no withdrawal period would make it a worthwhile treatment, especially for dairy farmers as it would allow them to sell the milk of implanted cows. For meat producing animals, an implant

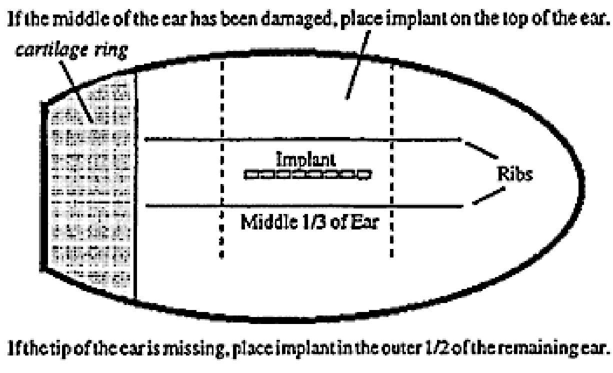

Figure 3

would be an advantage as the implant is placed in the ear (Figure 3) which is discarded at the slaughterhouse. It is also practical as it would be a single injection, therefore causing less distress to animal, farmer and vet, as well as meaning that it is easier to treat large numbers of animals. There is a risk of infection when implanting but this can be significantly reduced by keeping the surrounding area clean a and making sure the animal's ear is thoroughly disinfected. The implant would also ensure each animal got a complete course of treatment, which should reduce the mutation of the disease.

On the other hand, an siRNA implant would be expensive and it would be a big factor to weigh up the price of treatment with the value of the animal, especially with the question over a possible withdrawal period. It is also unknown how the treatment would affect pregnant animals and their young. Another problem with implants is the release mechanism. Most implants rapidly release when they are first injected into the animal, with the amount then falling to a threshold. This first rapid release could be potentially dangerous with siRNA, and it would take time for the implant to reach a threshold which would provide the required amount at the correct levels. It would also be difficult to monitor the levels of siRNA, and it may be required to change the amount needed half way through the implant's life, which would not be easily possible.

This does not solve the problem of mutating viruses either, or the fact that there are numerous different strains of the disease. It may be possible to inject different forms of the siRNA in one implant, but we do not know how they might react together and what side effects this would cause. Also the life of the implant would depend on the rate of mutation of the virus, as the implant may still release siRNA that would be useless as the virus has changed.

The various serotypes of bluetongue bring with them an ethical question. Different continents seem to be mainly affected by one or two serotypes of the disease, for example Europe seems to be mainly affected by the K and F serotypes. Should research money into curing a specific serotype be used for those prevalent in the developed world, like North America and Europe, or for those more widely found in the developing world, for example Africa and South America? Indeed, should the money go into research, or should it be put towards helping vaccinate against the disease in countries where farmers cannot afford to do so, and where they have far more to lose. In the opinion of many, prevention is far better than cure, but sometimes prevention is not completely effective or the disease becomes resistant to the vaccine, which in when we begin to rely more on the cure, therefore it could be said it is better to prepare for the future when this happens, then to find ourselves without prevention or cure.

Many people may have a problem with the research into the use of RNAi to cure bluetongue as it is not a zoonotic disease. They would feel that research money should go into looking for a cure for human diseases before animal, for example diseases such as cancer and

HIV/AIDS; although any research into RNAi as a cure would apply to a variety of different diseases. Others have a problem with animal testing, although animal activists may not have such a problem with animal testing to help other animals.

At the moment, bluetongue does not present a major threat to the UK, as long as the vaccination programme continues, but rising temperatures and increased travel, along with the chance of vaccine resistant strains of the disease developing, it is important that we continue to think about the possibility of curing it with RNAi treatment. RNA interference definitely has a future as a cure in the medical world for many diseases, however much more research is needed before we can think of using it on humans and animals.

References

Kennedy, A. (2008) OCR AS Biology, page 120 (Protein synthesis), Heineman.

The Mechanism of RNAi

http://www.ambion.com/techlib/append/RNAi_mechanism.html

Figure 1

http://www.ambion.com/figs/f00231.gif

The work of Andrew Fire and Craig Mello that led to their winning the Nobel Prize.

http://nobelprize.org/nobel_prizes/medicine/laureates/2006/press.html

The work of the UGA, College of Veterinary Medicine

http://www.vet.uga.edu/id/tripp/RNAi.php

Information and statistics on Bluetongue

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http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/bluetongue/about/index.htm

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http://school.eb.co.uk/all/eb/article-9063221#225291.hook

http://www.bbc.co.uk/scotland/outdoors/articles/bluetongue/

Figure 2

http://ec.europa.eu/food/animal/diseases/controlmeasures/bluetongue_restrictedzones-map.jpg

Information on Growth hormone implants and Figure 3

http://sustainabletable.org/issues/docs/Utah_University_Implants.pdf