

HOW THE MYXOMA VIRUS' MUTATION RATE EFFECTS
THE POTENTIAL TREATMENT OF MYXOMATOSIS
WHEN USING THE RNA INTERFERENCE MECHANISM

BY

JEMMA DUNKERLEY

PASS WITH MERIT

RESEARCH PAPER

BASED ON

PATHOLOGY LECTURES

AT VET-MEDLINK 2009

ABSTRACT

In this project I have looked at RNA interference, how it works and its possible uses. I have looked at specifically how RNAi research could be used as a possible, more effective treatment for Myxomatosis in rabbits, caused by the Myxoma virus. This has also made me look into possible problems; particularly the mutation rate of the Myxoma virus. My research has shown that an effective treatment could possibly be developed in the future via further research and testing. I believe that RNAi is a valuable tool and should not be ruled out as an alternative treatment for rabbits with Myxomatosis.

INTRODUCTION

The effects of RNA Interference (RNAi) were first observed in the early 1990's as experiments performed by plant scientists attempted to alter the colours seen in the flowers of petunias. Researchers introduced additional copies of the essential enzyme chalcone synthase (gene encoding for flower pigmentation) to petunia plants that were either of pink or violet colours. The expected result was to see darker flowers but in fact less pigmented, partially white or white flowers were produced.

Figure 1: Petunia plant's genes for pigmentation are silenced by RNAi



This indicated that the enzyme, chalcone synthase's activity had decreased, and that the petunias observed were as the result of post-transcriptional inhibition of gene expression due to an increased rate of messenger RNA (mRNA) degradation. This was called *co-suppression of gene expression*. However, the mechanism was unknown and was believed not to exist in animals.

On February 19th 1998, American scientists Andrew Fire and Craig C Mello published their findings in the journal "Nature". Their

discovery triggered a wave of excitement amongst fellow scientists, as the molecular mechanism they had discovered regulated the expression of many genes in multicellular organisms, including humans and animals; which led them being awarded the Nobel Prize for medicine in 2006. They had showed that gene silencing could be achieved in the nematode worm *Caenorhabditis elegans* by simply introducing short double-stranded RNA (dsRNA).

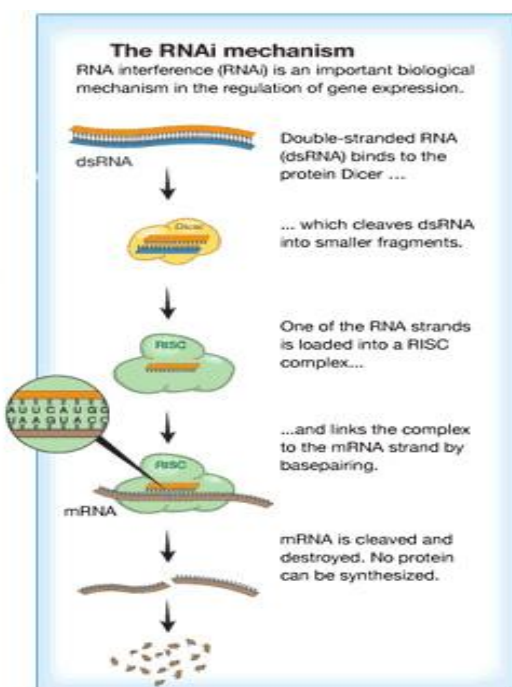


Figure 2: "The RNAi mechanism" shows how mRNA is degraded via a sequence of steps

Gene silencing occurs when double-stranded RNA (dsRNA) is cleaved by the protein complex, Dicer, making short fragments of approximately 20 nucleotides. Then another protein complex, known as a RNA-induced silencing complex (RISC), binds to one of the short fragments, known as small-interfering RNA (siRNA). This short fragment (siRNA) may also be called a guide strand. During this stage, the other RNA strand is eliminated, whereas the siRNA fragment bound to the RNA-induced silencing complex (RISC), detects mRNA molecules. The studied outcome is RNA interference (RNAi), where the guide strand (siRNA) attached to the RISC, base pairs with the complementary mRNA molecule. When this occurs, Argonaute proteins (enzyme), the catalytic components of the RNA-induced silencing complex (RISC), bind to the small-interfering RNA (siRNA) and are responsible for the breakdown of mRNA; via endonuclease activity. This means that proteins can no longer be synthesised, as the mRNA molecule will not reach the ribosome during the protein production sequence, and therefore prevents the expression of the gene (genes could be silenced).

With this in mind, it is not surprising that RNAi research has such a prevalent importance and relevance to veterinary medicine (and medicine). Current research includes looking at ways to fight diseases such as HIV, Hepatitis and Cancer (and many others). Because the HIV virus mutates rapidly, it has been harder to specifically target, as siRNA molecules are very short in length and specific. Also experiments that have proved successful in a laboratory may not have the same outcome in the body. This is also a problem that has been faced with RNAi Cancer therapies. A possible solution would be to use RNAi in association with other treatments to reduce resistance to drugs. Hepatitis provided evidence for the first use of RNAi as a therapy for diseases in animals and early studies showed that RNA silencing was found more prominent in the liver; which was ideal for therapeutics.

DISCUSSION

Future research possibilities, involving RNAi and veterinary medicine, could include finding an effective treatment for Myxomatosis; a disease that affects rabbits.

Myxomatosis was first discovered in 1869 in imported rabbits within Uruguay. It reached the UK in 1953 and by 1955 around 95% of the rabbits in the UK had died. However, resistance has been increasing slowly since the 1970's, and Myxomatosis now kills around 50% of infected rabbits.

The disease is spread by direct contact of an infected animal, or by being bitten by parasites, such as fleas or mosquitoes (vectors), that have fed on an infected rabbit. Due to the transmission of Myxomatosis via vectors, it is advised that animals that live in enzootic areas are vaccinated against the Myxoma virus (a poxvirus).

The vaccine used in Britain is called Nobi-vac Myxo, and it contains a harmless virus called Shope Fibroma. Antibodies made in response to the Shope Fibroma virus also protect rabbits from the Myxoma virus (Myxomatosis).

If the Myxoma virus is contracted in European rabbits (*Oryctolagus cuniculus*), the disease at first is usually visible by lumps (Myxomata) and puffiness around the head and genitals. This could then lead to acute conjunctivitis followed by possible blindness. Rabbits will develop a fever, lose their appetite and become listless.



Figure 3: European rabbit suffering from Myxomatosis

Not only this, diseased rabbits also incur secondary bacterial infections leading, in most cases, to suffering from purulent inflammation of the lungs and pneumonia. Death on average takes 14 days; however, it may take place in as little as 48 hours. In rabbits of the genus *Sylvilagus*, the Myxoma virus only causes localised skin tumours.

There are currently no treatments that are particularly effective for Myxomatosis. Treatments available include regular bathing, fluid therapy, feeding- via syringe or tube if necessary, antibiotics and careful nursing in a warm environment (21-22 °C). Unfortunately the commonest procedure with rabbits suffering from the disease is for them to be euthanased. However, there is now the possibility of developing a treatment for Myxomatosis through the use of drugs based on the discovery of RNAi.

RNAi relies on the siRNA to base pair with complementary mRNA. It is the siRNAs that can be made to silence almost any gene (or breakdown the mRNA of a virus). If long chains of dsRNA enter an animal (for example 30 nucleotides in length) it would lead to a complex interferon based inflammatory response. Therefore, short double strands, that are smaller than 23 nucleotides in length would be needed instead, as the siRNA would not trigger an inflammatory response, but will still trigger the interference response. This would mean that if a drug was to be developed to treat Myxomatosis, then the siRNAs used to bind to the Myxoma virus' mRNA would have be smaller than 23 nucleotides for RNAi to occur.

Short interfering RNAs (siRNAs) can be chemically synthesised. This is because siRNAs have a defined structure.

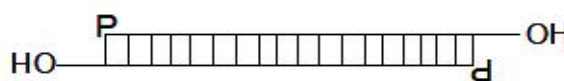
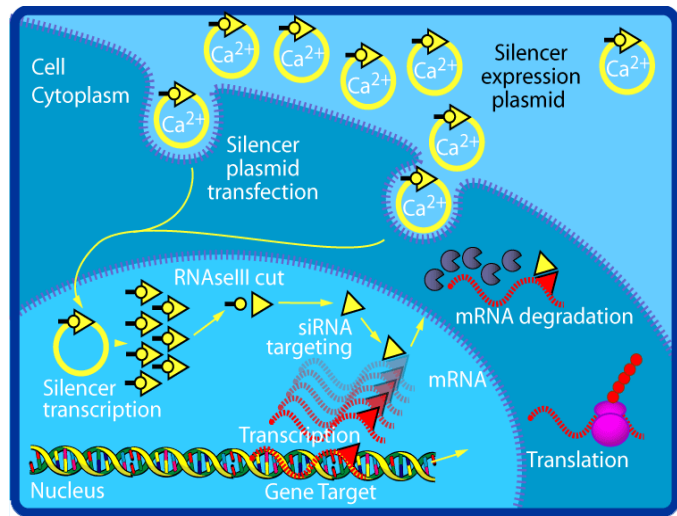


Figure 4: An example of a siRNA strand and its structure.

Schematic representation of a siRNA molecule: a ~19-21basepair RNA core duplex that is followed by a 2 nucleotide 3' overhang on each strand. OH: 3' hydroxyl; P: 5' phosphate.

Short interfering RNAs (siRNAs) can take part in RNAi-related pathways (rather than just their role in the RNAi mechanism). This may include an antiviral mechanism. Short interfering RNAs (siRNAs) can also be created in vitro transcription systems.

Figure 5: The RNA interference mechanism in cultured mammalian cells



If siRNAs can be introduced into a mammal (which they can if the siRNAs used are 23 nucleotides or smaller in length), then siRNAs must be able to be introduced into a rabbit that has contracted the Myxoma virus. As siRNAs can be chemically synthesised, in theory, the siRNA made will bind

complementary to the mRNA (of the Myxoma virus), and in turn could be used as an effective method to treat a rabbit suffering from Myxomatosis (by the degradation of the mRNA via RNAi). However, because the Myxoma virus mutates frequently this poses a problem when finding virus-specific siRNAs that will bind to the mRNA.

The Myxoma virus' genome is 161,773 nucleotides long, with a guanine and cytosine content of approximately 40%. It is liable to variation due to natural mutation. Natural mutation is also a common problem when developing vaccines for viruses such as Influenza as the virus mutates year after year (different strains, each with different receptors, means the drugs will also have to change).

There are a number of ways to solve the siRNA and the Myxoma virus' mRNA specificity problem. A possible solution would be to introduce many different synthesised siRNAs into a diseased rabbit, in hope that one of the siRNAs in the developed drug would be complementary to the Myxoma virus' mRNA (even if it has mutated). However, the lengths of these siRNA strands would have to be kept within the guidelines (smaller than 23 nucleotides in length) to prevent a complex interferon based inflammatory response, and the effects that these siRNAs would have on the rabbit, other than their role in RNAi would have to be monitored (such as vital cellular processes being impaired by siRNA strands that are not specific to the target Myxoma virus). The idea of using several siRNA strands would also have to be considered further, as combining the use of other treatments alongside would help prevent drug resistance.

Another idea (if you knew the most common strain of the Myxoma virus that year for example) would be to obtain strands of DNA, which once in the body will produce

siRNAs. Problems would occur with this method though, such as finding a way to keep the levels of siRNAs at a constant, plus the fact that there would not be variations of siRNA strands being produced to deal with possible mutations of the Myxoma virus. You would also need to carry out further research as to how the siRNAs would affect the diseased rabbits.

Several ethical issues to consider as a result of developing RNAi treatment might include any possible side effects of a developed drug, such as vital cellular processes becoming impaired by siRNA strands that are not specific to the virus' mRNA.

However, the most controversial ethical issue for the development of RNAi would be testing on animals. RNAi testing could lead to irreversible damage to cells, where animals could die or have to be euthanased. The advantages and disadvantages involving animal testing need to be considered thoroughly.

Currently there are three separate licences required to carry out animal testing. The Animals (Scientific Procedures) Act 1986 declare that animal procedures can only; take place in a suitable research institutes/ companies that have appropriate facilities and have be given a certificate of designation, be carried out by people with sufficient training and experience as seen in their personal licence and is part of an approved research/ testing programme that has been given a project licence. Without all three licences animal testing cannot take place. These licences are achieved only if; the minimum number of animals are used, the research cannot be carried out using non-animal methods, the potential results are important to warrant the use of animals, any suffering is kept to a minimum by suitable use of anaesthetics or analgesics and the necessary facilities and researches/ technicians are available.

The testing required for developing a treatment for Myxomatosis through the use of drugs based on the discovery of RNAi would have to comply with the Animals (Scientific Procedures) Act 1986. The main aim would be to solve the medical problem, in this case Myxomatosis. The research carried out should be done, only when absolutely necessary and aim to reduce the animals suffering as much as possible when developing the drug. Two possible procedures for researching the potential of the drug would include either; genetic modification to study the Myxomatosis disease or to test on rabbits that have already contracted the Myxoma virus. Both of these methods are highly controversial and animal welfare groups strongly question the effectiveness and reliability of any possible results.

The opposition against the use of animal testing usually feel that the ratio of loss and gain is unbalanced and that experimentation on animals is not worth the stress and possible cruelty that animals undergo. The UK Government annual statistics in 2007 revealed that over 3.2 million animals die in British laboratories and therefore animal

welfare groups are hoping that in the future alternate methods will be used, for example, computer generated testing.

However, even if testing was not initially carried out on animals, drugs would have to undergo trials using animals before it could be prescribed or used as a treatment within a veterinary practice, as experiments that have proved successful in a laboratory may not have the same desired outcome within the animal's body.

CONCLUSION

I have looked at how siRNAs and the RNAi mechanism could possibly be used to develop a drug to treat Myxomatosis. I have also considered the effect of the Myxoma virus' mutation rate.

Every day, our knowledge and understanding of genetics is expanding. Such as how DNA and RNA are expressed by the body. New techniques are also being discovered.

My research has showed me that developing a drug should be possible, based on current technology and understanding. If this is not possible now, I definitely believe that a drug could be developed to treat rabbits suffering from Myxomatosis in the near future.

There are still problems to overcome though. This includes developing a drug that will be able to cope with the mutations of the Myxoma virus and the chance of drug resistance when using several strands of siRNAs. I have suggested how to possibly solve these issues, but only by carrying out experiments and the use of animal testing would make these methods valid techniques.

In addition, there are controversial ethical issues based on animal testing and developing or working with an animal's RNAi mechanism with synthesised siRNA strands, even though the treatment possible could lead to less suffering and a possible development of a drug that could treat and cure rabbits that have contracted the Myxoma virus.

To conclude, RNAi is a powerful research tool. The uses of siRNAs in veterinary medicine (and medicine) are likely to play a vital role in the future. I have only discussed one potential application of siRNAs within this research project. However, I believe that the potential of siRNAs are still yet to be discovered. Well known diseases such as Cancer or even HIV would be a good avenue for future siRNA research, in hope to find a treatment. The future applications of the RNAi mechanism and the use of siRNAs are no doubt endless.

REFERENCES

1. <http://www3.interscience.wiley.com/journal/119069163/abstract?CRETRY=1&SERTRY=0>
2. <http://www.uncaged.co.uk/>
3. <http://www.esf.org/research-areas/medical-sciences/news/ext-news-singleview/article/new-treatments-for-viral-and-other-diseases-by-blocking-genes-444/news-browse/2.html>
4. http://www.ambion.com/techlib/tb/tb_506.html
5. <http://www.nature.com/horizon/rna/background/interference.html>
6. <http://www.esf.org/research-areas/medical-sciences/news/ext-news-singleview/article/new-treatments-for-viral-and-other-diseases-by-blocking-genes-444/news-browse/2.html>
7. <http://www.nature.com/gt/journal/v13/n6/full/3302703a.html>
8. http://upload.wikimedia.org/wikipedia/commons/2/24/Rnai_phenotype_petunia_crop.png
9. <http://upload.wikimedia.org/wikipedia/en/a/a9/SiRNAvitro.gif>
10. http://www.thehuntinglife.com/forums/uploads/monthly_02_2008/post-1-1203011969.jpg
11. <http://en.wikipedia.org>
12. <http://www.si-rna.com/>
13. <http://www.bio.miami.edu/~cmallery/150/nobel/RNAi.Nobel.htm>
14. <http://www.hhmi.org/research/nobel/mello.html>
15. <http://www.archive.official-documents.co.uk/document/hoc/321/321-xa.htm>