RNA interference:
Will the overlooked nucleic acid be the new star among animal health technologies?

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Introduction

The "central dogma" of modern biology, since its adoption in the mid-20th century, has been the basis for our understanding of how genetics impacts animal phenotypes. However, discoveries in the past 20 years have greatly complicated this simple storyline. Among these new discoveries, RNA interference is exciting not only because it allows us to more fully understand the inheritance of complex traits, but also because it is allowing for the development of an entirely new class of pharmaceuticals.

What is RNA interference?

In genetics, it is common to think of DNA as containing sequences that encode for proteins (genes) and a bunch of other DNA that is unimportant. This thinking is based on the central dogma of biology, which states that DNA serves as a template for RNA production, and RNA serves as a template for protein production, ultimately driving the form and function of the organism. Unfortunately, this elegantly simple paradigm has been muddied by the discovery of a number of very important non-coding RNA species. Scientists working with the roundworm in the early 1990s discovered short segments of RNA that did not code for protein at all (Lee et al., 1993). Instead, these segments (now called microRNA) could align with a longer RNA strand containing a complementary sequence, and cause it to be degraded, blocking protein production (Figure 1). This biological phenomenon was later verified to occur in many other species, including plants, animals, and humans.

Scientists began to realize the importance of this mechanism in normal development and function, partly because of the sheer number of genes that are regulated in this manner. The number of potential target genes varies between species, but recent estimates are that about 50% of human genes can be regulated by microRNA.

Further groundbreaking work, resulting in a Nobel Prize in 2006, was the discovery that introduction of synthesized double-stranded RNA into the cell could mimic the naturally occurring process, bind to RNA of a specific gene, and prevent protein production (Fire et al., 1998). Not only has this provided scientists with a superior research technique to determine the function of specific genes, but this technology also has exciting potential to be developed as a tool to treat disease or affect physiological function.

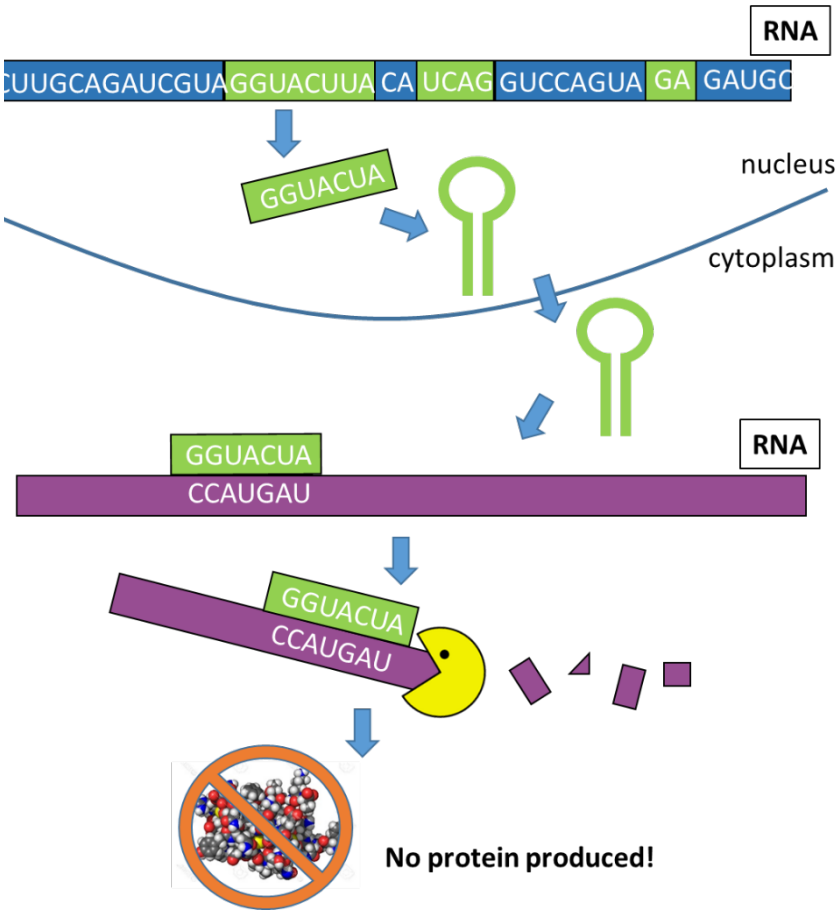

Figure 1. Mechanism underlying gene silencing by microRNA-mediated RNA interference. MicroRNA encoded by the genome is often found in introns that are excised from messenger RNA after transcription. The excised microRNA typically forms a hairpin loop that triggers post-transcriptional processing. The resulting short interfering RNA strand is complementary to a portion of a messenger RNA sequence, or often to several mRNA targets. The formation of double-stranded RNA causes the activation of an enzyme complex known at the RNA-induced silencing complex that degrades the mRNA. As a result, the protein encoded by the mRNA is not produced, resulting in a change in cellular function.

Micro RNA and inherited traits

One intriguing aspect of this new knowledge around RNA interference is the idea that introns (the non-protein coding "junk" DNA found within the coding sequences for proteins) may actually be the key genetic element underlying some selected traits. A single nucleotide polymorphism at a critical place within a microRNA sequence could, in theory, impact many proteins by creating or eliminating regulatory suppression by the microRNA through improved or impaired complementarity (Li et al., 2011; Li et al., 2015).

Epigenetics, though not the primary focus of this paper, has also turned traditional genetics on its head. The premise of this field of study is that environmental factors (i.e. diet, social interactions, physical activity) can lead to changes in animal function that can be passed on to the next generation, *without* an alteration in DNA sequence (Gonzalez-Recio et al., 2015). These

changes are typically driven by altered chemical states of DNA that influence its accessibility for transcription. Interestingly, microRNA play a key role in regulating epigenetic mechanisms (Holoch and Moazed, 2015), and therefore may be involved in inherited traits that are not encoded by DNA sequences. Conversely, epigenetic modification of DNA induced by butyrate (an end-product of ruminal carbohydrate fermentation) lead to alterations in microRNA expression (Li et al., 2010), suggesting a bidirectional link between epigenetics and RNA interference.

RNA interference as a next-generation pharmaceutical tool

In the same way that genetically-encoded microRNA can target mRNA sequences for destruction, synthetically-produced RNA molecules can be designed to silence proteins in a cell with great specificity (Bradford et al., 2016). This offers a number of exciting opportunities for addressing big problems in animal agriculture. For example, modern laboratory tools allow for the rapid genomic sequencing of new pathogens that can devastate livestock sectors; however, this information is currently put to use in a months-long process of creating vaccines. With continued developments in RNAi, it should be possible to rapidly (days or weeks) design and deploy small RNA molecules targeting the pathogen directly, allowing for a much more rapid counter-attack. Also, the active compound in RNAi is simply an RNA molecule (consumed daily by everyone), which makes this platform appealing from a food safety / residue perspective.

There are significant challenges to address before RNAi-based therapies will be practical for use in livestock. One hurdle that must be addressed for *in vivo* application of RNAi is the delivery of RNA molecules into cells of target organs. This is challenging because most cells do not normally take up RNA molecules. Secondly, the RNA molecules have to be protected from degradation while in transit to the organ of interest, which generally means using a nanoparticle with its own set of challenges. Finally, in light of recent survey data suggesting that 80% of consumers want mandatory labeling of food that contains DNA (Lusk, 2015), it is likely that consumers will initially be apprehensive about agricultural uses of RNAi.

Although the use of RNA interference to promote animal health may not come to fruition soon, it may be impacting human health already. The first phase 3 clinical trial utilizing RNAi is currently underway, targeting the elimination of a mutant protein that can cause a form of amyloidosis (http://clinicaltrials.gov/ct2/show/NCT01960348). More broadly, there is growing evidence that animal-source foods may impact human health in part by delivering biologically-active microRNA (Zempleni et al., 2015). Among the hundreds of microRNA found in cow's milk, one sequence known as miR-29b was shown to increase in circulation follow human consumption of the milk, and the downstream targets of this microRNA were altered in blood cells (Baier et al., 2014). Because miR-29b has been shown to promote the growth of cells that create bone matrix, these findings implicate cross-species RNAi as a likely factor in the beneficial effects of milk consumption on bone density. It is entirely possible that humans have been affected by diet-derived RNAi "nutraceuticals" for millennia!

Conclusions

We are in the very early stages of understanding the role of microRNA in inheritance of traits in livestock, and we are also likely decades away from seeing commercial application of

RNAi therapeutics. Nevertheless, the growing understanding of this fascinating biological process, and its implications across species, has opened up exciting possibilities in both fields.

Literature Cited

Baier, S. R., C. Nguyen, F. Xie, J. R. Wood, and J. Zempleni. 2014. MicroRNAs are absorbed in biologically meaningful amounts from nutritionally relevant doses of cow milk and affect gene expression in peripheral blood mononuclear cells, HEK-293 kidney cell cultures, and mouse livers. J. Nutr. 144:1495–1500.

Bradford, B. J., C. A. Cooper, M. L. TIZARD, T. J. Doran, and T. M. Hinton. 2016. RNA interference-based technology: what role in animal agriculture? Anim. Prod. Sci. In press. http://dx.doi.org/10.1071/AN15437

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391:806–811.

Gonzalez-Recio, O., M. A. Toro, and A. Bach. 2015. Past, present, and future of epigenetics applied to livestock breeding. Front. Genet. 6:305.

Holoch, D., and D. Moazed. 2015. RNA-mediated epigenetic regulation of gene expression. Nat. Rev. Genet. 16:71–84.

Lee, R. C., R. L. Feinbaum, and V. Ambros. 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854.

Li, C. J., R. W. Li, and T. H. Elsasser. 2010. MicroRNA (miRNA) expression is regulated by butyrate-induced epigenetic modulation of gene expression in bovine cells. Genet. Epigenetics 3:23–32.

Li, Q.-L., Z.-H. Ju, J.-M. Huang, J.-B. Li, R.-L. Li, M.-H. Hou, C.-F. Wang, and J.-F. Zhong. 2011. Two novel SNPs in HSF1 gene are associated with thermal tolerance traits in Chinese Holstein cattle. DNA Cell Biol. 30:247–254.

Li, A., J. Zhang, Z. Zhou, L. Wang, X. Sun, and Y. Liu. 2015. Genome-scale identification of miRNA-mRNA and miRNA-lncRNA interactions in domestic animals. Anim. Genet. 46:716–719.

Lusk, J. 2015. Food demand survey (FOODS) - January 2015. Available at http://jaysonlusk.com/blog/2015/1/15/food-demand-survey-foods-january-2015.

Zempleni, J., S. R. Baier, K. M. Howard, and J. Cui. 2015. Gene regulation by dietary microRNAs. Can. J. Physiol. Pharmacol. 93:1097–1102.