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RNAi: Applications in Vertebrate Pest Management

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Sequence-directed inhibition of protein synthesis by RNAi has potential as a means to control pest wildlife. Species specific by design, RNAi reduces impacts on nontarget species and the environment. Additional research advancing the field of RNAi-based management of vertebrate pest wildlife is timely.

Plant scientist Richard Jorgensen first described his observation of gene silencing in petunias in the early 1990s. Since that time, Andrew Fire and Craig Mello earned a Nobel Prize for their work in nematodes and coined the phrase RNA interference. Significant effort has been made in human pharmaceuticals to use RNAi in the treatment of numerous diseases with the first clinical trials for an RNAi-based human therapeutics coming only 6 years after its discovery. The appealing attribute of RNAi is that it mediates sequence-specific degradation of mRNA. Therefore, RNAi is used to block the synthesis of specific proteins (Box 1). This is useful for disease treatment, but also shows promise for the development of species-specific toxicants or reproductive inhibitors for the control of animal species. There has been significant progress applying RNAi technology for the control of insect pests [1,2]; in 2017, the US Environmental Protection Agency registered the first RNAi-based product targeting insects. Despite the potential diverse applications of RNAi technology in vertebrates for fertility control, invasive species eradication, and pest species management to protect human health and agriculture, little progress has been made in applying RNAi to vertebrate control.

Invasive and pest wildlife species cause significant damage to ecosystems and human infrastructure, spoil food stores, and eat or ruin food crops. On average, rodents damage or spoil 10–30% of food crops (>77 million tons) annually, enough food to feed 180 million people [3]. Mitigating these effects often depends on controlling animals using chemicals in toxic baits or fertility control agents. Criticism of these control methods is often focused on lack of efficacy, risks to nontarget species, and environmental burden. Therefore, the development of novel control technologies must be focused on species specificity and low environmental impact. Sequence-specific gene silencing via RNAi holds promise for effective management of pest wildlife. The silencing of genes using RNAi can be more cost-effective and humane with fewer nontarget effects than current approaches.

RNAi as Lethal Control

The ability to silence a single gene that is critical to life shows significant potential for the field of toxicant development. Toxicants currently used to control vertebrates often have significant effects in nontarget animals [4]. RNAi-based toxicants can be designed specifically to the target animal's genes, reducing risks to nontarget species. To achieve this, genes from the target animal are compared with the gene sequences of nontargets to identify regions of significant divergence. RNAi molecules are then designed to bind those regions of the target animal's gene (Figure 1). These sequence differences result in a mismatch of the RNAi and the mRNA of the nontarget animal; this mismatch prevents binding and the subsequent gene silencing. Differences of a single nucleotide have been shown to be sufficient to prevent RNAi binding [5]. RNAi sequences are approximately 21 base pairs in length so the region to which the RNAi is designed can be moved down the gene until a region with no similarity to nontarget species is found. This enables researchers to develop RNAi molecules that have effects in target species with no effects in nontarget animals even if they are exposed to RNAi.

RNAi in Rodent Eradications on Islands

Invasive rats and mice cause significant harm to island ecosystems by damaging flora and causing the extinction of many species [6]. Fortunately, after the removal of invasive rodents, island ecosystems can flourish; however, these rodent eradications rely on the use of anticoagulant rodenticides and therefore pose risks to nontarget species. RNAi-based rodenticides would be an important innovation

Box 1. RNAi

RNAi is a biological mechanism that directs the post-transcriptional silencing of protein synthesis. It is an evolutionarily conserved mechanism in eukaryotes. RNAi is dependent on a protective pathway within cells that degrades mRNA and is thought to be evolutionarily maintained to protect against viral infections. Double-stranded RNA (dsRNA) binds to an enzyme called DICER that cleaves the dsRNA into smaller fragments which are then integrated into the RNA-induced silencing complex (RISC). The RISC complex is responsible for separating the short dsRNA fragments into single-stranded segments. One of these single-stranded segments is then shuttled to and binds the matching mRNA sequencing that was transcribed from DNA. The complex formed by the RISC (with fragment of the initial dsRNA) and the mRNA then cleaves and degrades the mRNA. The gene for which the mRNA coded is, therefore, not synthesized and the gene is silenced.

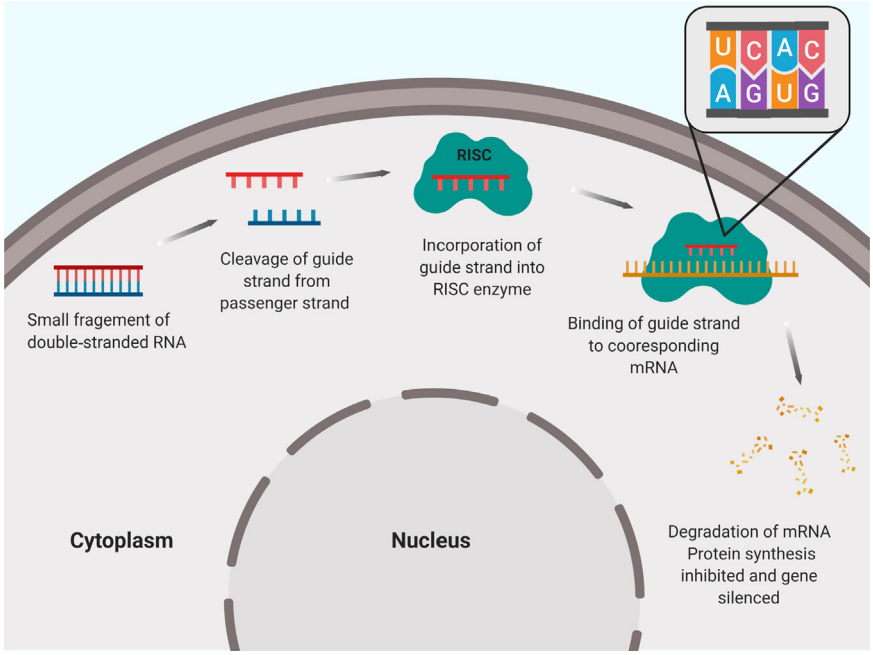

Figure 1. Pathway of RNAi Gene Silencing.(i) Double-stranded RNA fragment, 21–23 nucleotides in length in the cytoplasm of target cell following directed delivery by carrier molecule. (ii) The guide strand and passenger strand are separated. The passenger strand is discarded. (iii) The guide strand is incorporated into the RNA induced silencing complex (RISC). (iv) The RISC/guide RNA complex finds the corresponding target mRNA and binds. (v) mRNA is cleaved and degraded. The protein coded for by that mRNA is not synthesized. This is termed gene silencing. Figure created with BioRender.com.

and a significant advancement for island eradication efforts [7]. The costs associated with protecting nontarget species from harm represent a barrier to implementation of eradications on islands that house some of the world's most endangered species. Since RNAi-based rodenticides would pose little risk to nontarget species, this technology would enable rodent eradications on islands previously deemed to have species assemblages that were too complex for cost-effective eradication efforts.

RNAi to Disrupt Fertility

Although lethal control using chemical toxicants is a mainstay for the management of pest wildlife, inhibiting reproduction may be an effective means to control populations and might be preferred over lethal control in some cases. An inexpensive, noninvasive method to sterilize animals would be of benefit in the effort to manage both wild horse and feral dog populations without the use of lethal control. RNAi could be effective as a reproductive inhibitor for the control of feral animals. Silencing the *Kiss1* gene using RNAi has shown potential in initial studies [8,9] and meets many of the criteria for success. The *Kiss1* gene has limited distribution and is found in tissues involved in reproduction. Animals lacking *Kiss1* gene products (proteins) are infertile. Introducing RNAi for the *Kiss1* gene would block the synthesis of the kisspeptin protein that is critical to reproduction. The systemic delivery of an RNAi-based reproductive inhibitor that causes permanent sterility would help in the nonlethal management of many wildlife species, reducing not only the damage they may cause to the environment and human infrastructure but also the transmission of zoonotic diseases.

Risks and Benefits

Although RNAi is often grouped together with gene drives in discussions of risk [10], there are significant differences in the technologies. Gene drives increase the frequency of a specific gene over time in a population through genetic modification that is passed down to offspring [11]. RNAi molecules do not need to be incorporated into the genome of animals to be efficacious. Therefore, RNAi does not genetically modify organisms. This is an important distinction because the hurdles of containment associated with gene-drive based approaches do not apply. Risk evaluation for RNAi-based tools (toxicants or reproductive inhibitors) could likely follow the current paradigm for traditional chemical toxicants. That is, if unpredicted risk from RNAi-based tools is found, they could be removed from use until mitigation measures or further technological advancements are made. However, if current risk analysis techniques are to be used, careful selection of delivery platforms should be made as virally vectored RNAi tools may have different risk profiles. The benefits of RNAi-based tools are far reaching, as the risks to the environment and nontarget species are minimized, allowing for widespread use.

Concluding Remarks

Despite the potential diverse applications of RNAi technology in vertebrates (fertility control, invasive species eradication, and pest species control to protect human health and agriculture), little progress has been made in applying RNAi to these classes of animals. A single proof-of-concept study using RNAi to control sea lampreys combined with recent advances in RNAi delivery have opened the door to numerous innovative applications of this technology in vertebrate management [12]. One of the hurdles to further development of RNAi in vertebrates will be delivery. To facilitate widespread use of RNAi-based

products, oral baits will likely be necessary. Delivering RNAi orally will face many challenges, including protection from the hostile conditions in the gastrointestinal tract (GI), uptake into the systemic circulation, and shuttling the RNAi to the site of action. Research in nanotechnology has led to the development of numerous different nanoparticles that have been shown to facilitate effective oral delivery and GI uptake [13]. Other scientists are making progress tackling cellular delivery and internalization using cell-penetrating peptides [14]. Continued research in delivery, *in vivo* shuttling, and cell penetration will be imperative for the success of RNAi technology in vertebrate pest management applications.

Because they are species-specific by design, RNAi-based products reduce risks to nontarget organisms, enabling them to be used broadly across landscapes, even in ecosystems that historically have been avoided because of environmental concerns. However, the small segments of nucleotides that make up RNAi molecules may still be recognized as foreign by both target and nontarget animals, thereby eliciting an immune response. These possible immune adverse effects of RNAi will need to be considered during RNAi development. Advances have been made toward reducing immune activation but continued research is important to ensure low risks to nontarget species [15]. Another benefit of interfering RNA is that it does not involve modification of the genome and consequently is not heritable; therefore, it is free from the controversy surrounding gene drives. Continued research in RNAi will provide breakthrough advancements in tools to control animal species protecting human health, agriculture, and infrastructure. Their use on the landscape level will require careful development of baits that retain activity through exposure to the conditions of the GI tract and are stable in harsh environmental conditions of varying temperature, humidity, and UV exposure.

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