

# Insights into RNA Interference as Antiviral Defense

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## Abstract

The beginning of this century is marked by discovery of “RNA interference (RNAi)” and led to Nobel Prize award in Physiology or Medicine jointly to Andrew Z Fire and Craig C Mello in 2006. Initially discovered as innate antiviral defense mechanism in plant it has great implications as therapeutic tools to combat with animal and human viruses as well as research tool to study disease pathogenesis. This article reviews the mechanism of RNAi and outlines recent research directed toward development as antiviral defense in human and animal diseases.

**Keywords:** Antiviral; HCV; HIV-1; RNAi; miRNA; siRNA; siRNA delivery

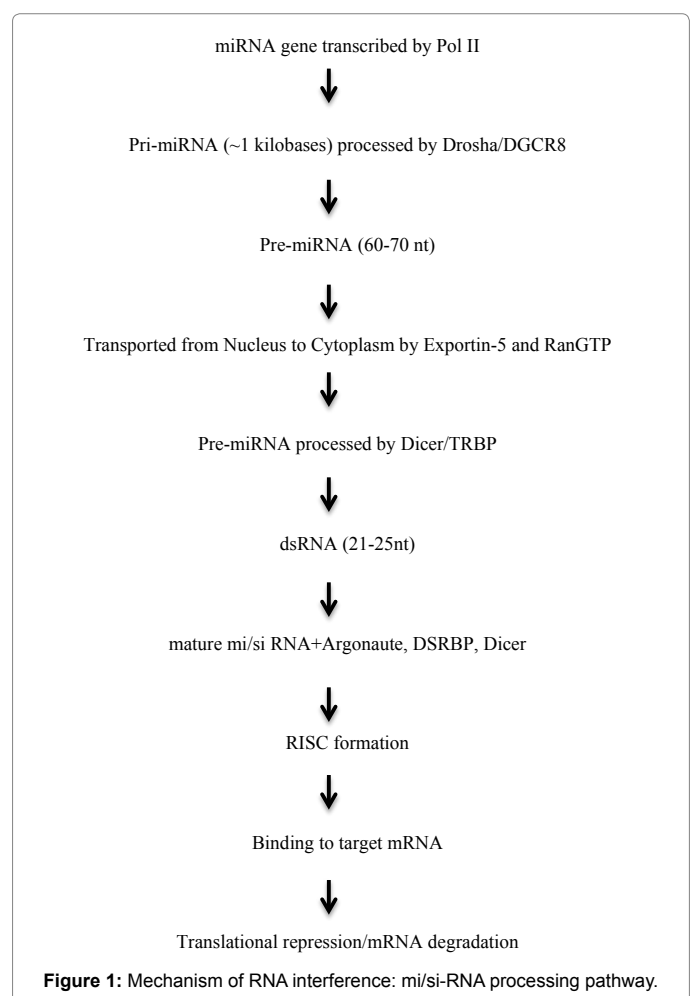
## Introduction

RNAi or double stranded RNA (dsRNA) dependent post-transcriptional gene silencing is evolutionarily conserved cellular mechanism thought to be evolved as defence mechanism to protect the organisms against viral infections. Fire and Mello discovered that introduction of dsRNA resulted in profound specific gene silencing in adult animals and this effect is vertically transferable into progeny [1]. This phenomenon occurs in wide variety of eukaryotic organisms by generating small 21-25 nt single stranded RNA (ssRNA) from exogenously introduced dsRNA or endogenous RNA through series of processing by nucleases and cellular proteins, which leads to sequence-specific binding to messenger RNA (mRNA) and its silencing [2]. There are several noncoding RNAs described in eukaryotic organisms including microRNA (miRNA), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), long noncoding RNAs (lncRNAs) and small interfering RNA (siRNA) [3,4]. The phenomenon of RNA interference is widely accepted as antiviral defense as well as molecular probes to investigate the viral pathogenesis. This article discusses the mechanism of RNAi in animal cells by endogenous as well as exogenous dsRNAs and their implication as tool to combat with viruses.

## Mechanism of RNA Interference

The major player of RNAi is 21-25 nt ssRNA which binds sequence-specifically to mRNA with help of argonaute-family ribonucleoprotein complex known as RNA-induced silencing complex (RISC). Depending upon the percent complementary of with target mRNA either this binding leads to degradation or translation repression of target mRNA. This ssRNA is generated from both exogenously delivered dsRNA and endogenous long ~ 1 kilobases RNA transcript known as primary miRNA (pri-miRNA) through similar mechanism [4-7]. In endogenous pathway, the pri-miRNA is cleaved into small 60-70 nt pre-miRNA by microprocessor complex composed of Drosha and DGCR8 [8] Then resulting pre-miRNA is transported from nucleus to cytoplasm with help of Exportin-5 and RanGTP [9]. In cytoplasm both endogenous pre-miRNAs and exogenous siRNAs follows same pathway by dicer enzyme and processed into 21-25 nt dsRNA [4,10]. With help of argonaute, dsRNA-binding protein (DSRBP) and dicer, this dsRNA forms RNA induced silencing complex (RISC) and one of the strand of dsRNA is degraded. Then argonaute and ssRNA direct this complex to target mRNA which leads to silencing of expression of target mRNA [10-12]. This is illustrated in Figure 1.

A wide variety of tools including web-based algorithms and



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software's are available to design of effective siRNA targeting specific genes [13-16]. Then these exogenous siRNA are synthesized by chemical synthesis, *in vitro* transcription or transfection of plasmid/viral vectors containing siRNA sequences for expression in cells [17-19].

## RNAi in Antivirus Defense

Since last one decade, several studies have reported use of RNAi to inhibit replication of retroviruses, respiratory syncytial virus, influenza virus, poliovirus, herpes viruses and hepatitis c virus. These studies have used either synthetic siRNA or expressed shRNA delivered via transfection or transduction [20-26]. The synthetic and *in vitro* transcribed siRNA targeting HIV-1 gag, regulatory proteins such as Tat and rev, resulted in strong inhibition of both T- and M-tropic HIV replication [27-31]. Moreover, siRNA targeting essential host cell factors for HIV-1 replication effectively inhibits HIV-1 replication in cell culture [30,32]. Similar to HIV-1, targeting influenza virus essential genes including PB2, M2 and NP resulted in strong inhibition of virus in cell culture [33-35].

In addition to these synthetic exogenous siRNA, the endogenous human miRNAs also play important role in viral pathogenesis. The human mir-532 inhibits West Nile Virus indirectly by suppressing expression of host proteins TAB3 and SESTD1 [36]. Similarly, mir-29 family plays important role in decreasing HIV-1 viral load, progression to AIDS as well as boosting host immunity [37]. Moreover, several miRNAs play important role in HIV-1 pathogenesis either by directly inhibiting viral genes or host cell factors important in viral pathogenesis [38]. Hariharan et al. using target prediction softwares demonstrated that four human mir-29a/b, mir-149, mir-378 and mir-324-5p targets *nef*, *vpr*, *env* and *vif* genes respectively [39]. The group of human miRNAs including miR-28, miR-125b, miR-150, miR-223 and miR-382 targets 3' end of HIV-1 mRNAs and significantly decrease HIV-1 replication in n resting CD4<sup>+</sup> T cells [40]. Similarly, mir- let-7c, mir-192 and mir-142 inhibit influenza virus replication [41-43].

The siRNA even delivered *in vivo* in infected animals inhibited Japanese encephalitis virus in mice, HBV and influenza virus in transgenic mice [44-46]. The HIV-1 core as well as accessory proteins and several essential host genes used as RNAi target for HIV-1 inhibition *in vivo* using humanized mice infected with HIV-1. These studies showed significant reduction in viremia and infection associated syndromes in mice as well as resistance to HIV-1 infection in humanized BLT mice [47,48]. The siRNA targeting NS1 gene of influenza strongly inhibited virus replication in mice [49].

## Delivery of Small RNA

The siRNAs are delivered to cells by transfection of synthetic or plasmid expressing siRNA using cationic liposomes, polyamines as well as electroporation and viral transduction. This delivery can be enhanced with chemical modification by conjugation with bioactive moieties and nanoparticles [50]. Moreover, negative charge of siRNA enabled the use of cationic peptide such as including poly(L-lysine) (PLL), protamine and cell penetrating peptides (CPP) as a efficient and stable carrier for delivery [51]. Further research demonstrated that gold nanoparticles as efficient vehicles for siRNA delivery *in vitro* and *in vivo* as well as enhances stability [52]. Recently, Duan et al. demonstrated efficient delivery of siRNAs to the liver for targeting hepatitis C virus using vitamin E and cholesterol-based cationic liposomes (VE-DC) without toxicity [26].

## Conclusion

RNA interference is innate, sequence-specific antiviral defense mechanism in plants and lower eukaryotes and this can be potentially used as tool against animal and human viruses. These small single stranded RNA can be designed to target specific gene important for viral replication and pathogenesis. Recent research highlights the discovery of endogenous miRNA inhibiting viral infection. This si/ miRNA clears viral infection by directly inhibiting target genes without causing adverse effect on host cells. This beauty of RNAi kindles hope of developing an effective therapeutics against deadly viral diseases.

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