

SILENCING VIRUSES BY RNA INTERFERENCE

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Abstract

Post-transcriptional gene silencing (PTGS) makes possible new approaches for studying the various steps of the viral cycle. Plus-strand RNA viruses appear to be attractive targets for small interfering RNAs (siRNAs), as their genome functions as both mRNA and replication template. PTGS creates an alternative to classic reverse genetics for viruses with either negative-strand or double-stranded RNA genomes and for those with a large genome. PTGS allows modification of the expression of a given cellular gene as a means to elucidate its role in the viral cycle and in virus-host cell interactions, and to investigate cellular pathways involved in viral pathogenesis. It also allows the creation of new animal models of human diseases. In addition, PTGS already appears to be a promising new therapeutic tool to fight viral multiplication and dissemination through the host and to prevent inflammation and virus-induced pathogenesis, including virus-induced tumorigenesis.

Introduction

Small noncoding RNAs have important roles regulating a wide range of cellular pathways associated with developmental programs and the protection of the genome against mobile genetic elements. In particular, small interfering RNA (siRNA) molecules mediate post-transcriptional gene silencing (PTGS), a natural biological phenomenon discovered in plants [1] and *Caenorhabditis elegans* [2]. In plants, it is a natural, conserved mechanism of antiviral immunity. PTGS is mediated by siRNAs produced by the type III endoribonuclease Dicer processing double-stranded RNA precursors [3]. T. Tuschl and coll. [4] demonstrated that synthetic 21 nucleotide (nt)-long RNA duplexes transfected into cultured cells silenced genes in a sequence-specific manner. These synthetic siRNAs, with 2 nucleotide 3' overhangs, mimic those produced by the ribonuclease Dicer by digestion of large double stranded (ds) RNAs. Duplexes of RNAs shorter than 30 base pairs (bp) generally do not activate non-specific suppression by double stranded (ds) RNA-dependent protein kinase [4,5]. After incorporation into the RNA-induced silencing complex (RISC), one of the siRNA strands acts as guide and selects the homologous target in the mRNA, which is then cleaved and degraded by RNases (Fig. 1). The efficiency of siRNAs is generally greater than that of short antisense RNAs. One characteristic of siRNAs is that for activity, they must be perfectly base-paired to their target in the corresponding RNA. However, up to 3 G:U wobble base pairs do not alter target specificity [6], probably because they have the same thermodynamic stability as Watson-Crick base pairs [7]. Other small duplex RNAs, named micro RNAs (miRNAs), are also dependent on Dicer for processing, but do not require perfect complementarity of the mRNA target and block gene expression through translational inhibition [8,9]. This mechanism therefore differs from that of PTGS, which is highly sequence-specific. It has been recently shown that after transfection, small duplex RNAs

displaying one or few mismatches with their target sequence sometimes behave like miRNAs, inhibiting translation of the mRNA, which remains intact [9].

Preferably, siRNA target sequences should be chosen outside of RNA regions predicted to be highly structured [4]. Guidelines for the choice of siRNA sequences have been published [10-12]; they include a G:C bp at the 5' end of the sense strand of the siRNA, an A:T bp and an A-T rich sequence at the 5' end of the antisense strand of the siRNA, and no G-C stretch longer than 9 bp. Enzymatically prepared siRNAs have been synthesized in several ways to improve their efficiency and to lower the cost. In one approach, a large duplex RNA is cleaved *in vitro* by a cloned Dicer, as occurs in cells [13]. This has the advantage of producing a large panel of siRNAs covering a long segment of the mRNA, thereby enhancing efficiency and, in the case of viruses, avoiding the emergence of escape mutants. Another method involves using the T7 polymerase to transcribe siRNAs from short DNA templates encoding siRNAs downstream from a T7 promoter [14]. One of the limitations of chemically or enzymatically synthesized siRNAs is their transient effect in transfected animal cells. Chemically-protected siRNAs and viral vectors have been used to circumvent this problem [15-17].

The siRNA technology is so powerful that it is leading to a revolution in molecular virology. It makes possible new approaches for studying the various steps of the viral cycle, not only for plus-strand RNA viruses (Fig. 2), but also for all kinds of viruses. PTGS creates an alternative to classic reverse genetics for viruses with either negative-strand or double-stranded RNA genomes and those with a large genome. PTGS allows modification of the expression of a given cellular gene as a means to elucidate its role in the viral cycle and in virus-host cell interactions, and to investigate cellular pathways involved in viral pathogenesis. It also allows the creation of new animal models of human diseases. In addition, PTGS already appears to be a promising new therapeutic tool to fight viral multiplication and

dissemination through the host and to prevent inflammation and virus-induced pathogenesis, including virus-induced tumorigenesis (see below). The literature on PTGS is already substantial and this review is far from exhaustive. A few examples have been chosen among RNA and DNA virus families to illustrate the possibilities already offered by this new technology. Most retroviruses will not be considered because they will be the subject of a separate review in this journal.

SiRNA transfer and siRNA-encoding vectors.

SiRNAs are negatively charged and do not readily cross cell membranes. They can be introduced into cells by electroporation. Alternatively, delivery vehicles of two classes can be used: synthetic carriers such as cationic lipids, peptides and cationic polymers (reviewed in [18]); and viral vectors. Non-viral carriers present fewer safety concerns than viral vectors, but are generally less effective for delivery.

The effect of both chemically and enzymatically-synthesized siRNAs is generally transient and typically does not last more than a week [19]. To prolong the effects, RNA polymerase III-driven small RNA expression vectors have been constructed to induce long-lasting RNA silencing in mammalian cells [20]. SiRNAs are transcribed from H1 or U6 RNA polymerase III promoters through to a stretch of five T residues acting as a transcription termination signal. Positive and negative-strands of siRNAs are obtained from either tandem-type or hairpin-type transcripts. These constructs have been inserted into several types of vectors, including lentivirus, adenovirus and adeno-associated virus (AAV) vectors [16,17,21]. Long-term expression of si- or shRNAs, lasting several weeks, has thereby been obtained. Note that lentiviruses are suitable vectors for use in non-dividing cells, and in particular in certain primary cell cultures [22]. Episomal vectors have also been designed. One of them contains the ori P origin of replication, a coding sequence for EBNA-1 protein that is

required for episome maintenance and two H1 tandem promoters that drive the synthesis of each of the siRNA strands [23].

Methods commonly used *in vivo* include electroporation of siRNAs into the neural tube of chick embryos [24] and hydrodynamic injection into the tail vein of mice [25]. SiRNAs have also been delivered intranasally [26] and subcutaneously [27]. Viral vectors are routinely used *in vivo*.

SiRNA targets in the genome of plus strand RNA viruses.

Most regions of viral genomes have been assayed as targets for PTGS. Plus strand RNA viruses appear to be attractive targets for siRNAs, as their genome functions as both mRNA and replication template [28]. A pioneer study by Gitlin et al. [15], targeting the capsid protein region or the 3D RNA polymerase region of the poliovirus (PV) genome, has shown that the viral yield of a one step growth cycle was about 100-fold lower when cells were transfected by specific antiviral siRNAs before infection. Antiviral siRNAs interfered with viral multiplication early after infection, but were not dependent on viral replication, and induced specific post-transcriptional cleavage and degradation of viral RNAs. Cell protection was slightly extended when both anti-capsid and anti-polymerase siRNAs were used together. Neither interferon (IFN) nor either of its dsRNA-activated effectors, PKR or RNase L, was required for suppression of PV replication by siRNAs. Interestingly, escape PV mutants rapidly emerged when cells treated with single siRNAs were infected at a high multiplicity of infection (MOI=10) while they rarely emerged at lower MOIs, suggesting that they were already present in the initial viral population [15].

A DNA-based human hepatitis A virus (HAV) replicon has been used in a hepatoma cell line to investigate whether HAV replication is inhibited by siRNAs targeting regions of the replicon encoding nonstructural proteins [28]. Specific inhibition but not complete

suppression of HAV replication was demonstrated. As for PV [15], combinations of siRNAs were more effective than single siRNAs for suppressing viral replication [28]. Unexpectedly, two HAV-specific siRNAs induced increased replicon expression [28]. Thus, PTGS was ineffective in this case, probably because of the secondary structure of target RNA that may influence the efficiency of PTGS.

A similar phenomenon has also been observed in the case of a human hepatitis C virus (HCV) replicon and an siRNA specific for the first nucleotides of the HCV 5' noncoding (NC) region, which exhibits a stem-loop structure [29]. Given the medical importance of HCV infections, several laboratories have investigated the possibility of inhibiting the replication of HCV in cell lines of hepatic origin [23,30-32]. Since no efficient cell culture system for growth of HCV is available, these studies were performed with HCV replicons harboring a reporter gene and/or a selective marker. Only some of the HCV-specific siRNAs were effective (inhibiting HCV replication at least 10 fold) in an IFN- and cell cycle-independent manner [30]. A protocol involving two to three successive transfections with endonuclease-prepared siRNAs was highly effective [33]. In mice, an siRNA targeting the viral polymerase NS5B region reduced expression from a HCV NS5B-luciferase chimera by 75%, and inhibition was more than 90% when DNA templates encoding small hairpin RNAs (shRNAs) were used [34]. In a hepatoma-derived cell line that already harbors autonomously replicating subgenomic HCV RNA, luciferase activity was reduced by up to 85% in a dose-responsive manner after transfection of cells by a specific siRNA targeting the 5' NC region of HCV [31]. Prolonged siRNA effects were obtained with bicistronic plasmids expressing complementary siRNAs [23]. Wilson et al. [23] used a self-contained episomal expression vector that extended the duration of siRNA activity to three weeks. Introduction of siRNAs targeting another nonstructural protein, NS5A, resulted in the inhibition of NS5A and NS5A-mediated activation of the IL-8 promoter, as well as the inhibition of core protein expression

[32]. siRNA targeting the core region also resulted in dose-dependent and specific HCV RNA silencing [35]. siRNAs reduced the levels of both (+) and (-) strand RNA [36] and could clear replicating HCV RNA from >98% of cells. The HCV NS3-1 gene also appears as a good target for PTGS [37].

Dengue virus (DENV) is another plus strand RNA virus of great medical importance: it causes dengue hemorrhagic fever and it is responsible for dengue shock syndrome. AAV vectors encoding siRNAs specific for a 3' NC sequence common to all dengue serotypes reduce dengue infection in dendritic cells and decrease the dengue-induced apoptosis of these cells [38].

A novel coronavirus (CoV), responsible for severe acute respiratory syndrome (SARS), spread worldwide in early 2003. This virus (SARS-CoV), like other members of the *Coronaviridae* family, is enveloped and has a large genome of positive polarity. siRNAs targeting the SARS-CoV RNA polymerase blocked cytopathic effects of the virus on Vero cells, blocked viral RNA and protein synthesis, and reduced virus production [39].

siRNAs: an alternative to classic viral genetics.

Molecular genetics is difficult with viruses having either a negative-strand RNA genome, or a segmented genome, or a very large genome. For these viruses, the siRNA technology offers a potentially valuable alternative strategy to modulate viral gene expression.

Negative-stranded RNA viruses include important human pathogens. Among them, pediatric disease caused by human respiratory syncytial virus (hRSV) claims about a million lives annually. The nonsegmented genomic and antigenomic RNAs of hRSV escape siRNAs, probably because they are tightly wrapped with the nucleocapsid protein N, which makes them inaccessible [40]. Unlike the full-length genomic and anti-genomic RNA, the mRNAs do not bind N protein. Two types of hRSV-specific siRNAs have been designed: the first is

specific for the P protein, involved in the formation of a complex responsible for sustained RNA elongation during transcription. The second is specific for the fusion protein F. At nanomolar concentrations, the siRNAs specific for viral P and F mRNAs abolished expression of the corresponding mRNAs and proteins, and produced the expected mutant phenotype. Targeting the P mRNA substantially diminished all viral protein production, reduced viral progeny yield by up to 10^4 fold, and inhibited cell fusion. In contrast, targeting the F mRNA did not inhibit cytopathic effects completely but prevented syncytia formation. Therefore, hRSV-specific siRNAs allow functional genomic studies, attenuated infection, reverse genetic analysis and studies of host-virus signaling pathways [40].

Influenza viruses are another group of major human pathogenic viruses causing particularly severe respiratory infections in the elderly and immunocompromized individuals. One of the advantages of an siRNA treatment is that it does not require a functional immune system [41]. The genome of influenza virus A is composed of 8 RNA segments of negative polarity. Ge et al. [41] designed 20 siRNAs targeting regions of the viral genome that are conserved among different subtypes and strains of virus from human and animal species. Most viral genes were targeted, except the hemagglutinin and neuraminidase genes, because of the extensive diversity in these genes. SiRNAs targeting nucleocapsid protein NP and two proteins of the transcriptase complex (PA and PB1) were effective, even when cells were infected with virus a few hours prior to siRNA introduction. In addition, siRNAs inhibiting influenza virus production *in vitro* also inhibited virus production in chicken embryos [41]. Interestingly, the same authors demonstrated that the target of siRNA was either mRNA, or complementary (+) strand cRNA, or both, but not viral (-) strand RNA. However, targeting NP and PA genes resulted in overall inhibition of viral RNA transcription. Influenza virus matrix protein-encoding RNA has also been successfully targeted [42].

Rotaviruses are the leading etiologic agents of severe diarrheas in young children worldwide. They consist of three concentric layers of protein that enclose a genome of 11 segments of double-stranded (ds) RNA. Dimers of VP4 form 60 spikes at the surface of triple layered particles (TLPs) that are involved in virus attachment to the cell surface. SiRNAs corresponding to the VP4 gene efficiently inhibited VP4 synthesis, and the synthesis of other viral proteins was not affected. Most of the viral particles were TLPs, but lacked VP4. The results reported by Dector et al. [43] indicate that VP4 was not required for the budding of double-layered particles (DLPs) into the endoplasmic reticulum or for the enveloped intermediate particles to lose their lipid envelope. VP4 was not required for the assembly of VP7 into the third protein layer, but VP7 assembled in a loose manner under these conditions, and formed spike-less TLPs. The resulting particles were poorly infectious. These findings confirm the essential role of VP4 in the infectivity of rotavirus [43]. Interestingly, the viral transcripts involved in virus genome replication do not seem to be susceptible to siRNAs [44,45]. They may either be protected within electron-dense inclusions (viroplasms) or by RNA-binding proteins in the cytoplasm. Synthesis of VP4 was silenced even when the corresponding siRNA was added 4 h post-infection [44]. Silencing the VP7 gene prevented the formation of TLPs, and caused DLPs to accumulate [45]. Silencing the viral polymerase, VP1, and the nonstructural protein, NSP5, blocked the secondary transcription of the virus, with the concomitant inhibition of the synthesis of all viral proteins (reviewed in [44]). Another potential target is NSP4, since this viral enterotoxin contributes to diarrhea (reviewed in [44]). In the absence of a reverse genetics system, PTGS should be very useful for functional genomic studies in rotaviruses [44].

Herpesviruses are large DNA viruses that typically infect their host latently after an acute phase. Reactivation of the lytic virus cycle may occur following induction by various agents. Two human gammaherpesvirus, Kaposi's sarcoma associated herpesvirus (KSHV)

and Epstein-Barr virus (EBV), are associated with several types of malignancies. Jia and Sun [46] targeted the Rta gene of a murine gammaherpesvirus, herpesvirus 68 (MHV68), encoding an immediate-early viral protein responsible for the switch between the latent and lytic phases. They also targeted ORF45, a gene that is conserved among all gammaherpesviruses making it a good target. Anti-Rta siRNA cotransfected with virus DNA into permissive cells significantly blocked Rta expression. It also blocked the expression of other viral proteins. Cells cotransfected with either anti-Rta- or anti ORF45-siRNA grew better than controls and produced only 1 to 2% of the virus progeny at 4 days post-transfection [46]. Herpes simplex virus type 1 (HSV-1) is an alphaherpesvirus that infects epithelial and neuronal cells. Glycoprotein E (gE) from HSV-1 is essential for cell-to-cell spread. When human keratinocytes were transfected with gE-specific siRNAs and then infected with HSV-1, Bhunyan et al. [47] observed small plaques, corresponding to the phenotype of a gE-deletion mutant of HSV-1.

The human polyomavirus JC, which is responsible for a demyelinating disease (progressive multifocal leukoencephalopathy), has been successfully blocked by PTGS in human astrocytic cells [48].

Hepatitis B virus (HBV) causes both acute and chronic infection of the human liver, which can lead to the development of liver cirrhosis and hepatocellular carcinoma. In stable HBV-producing hepatoma cell lines, siRNAs targeting the polyadenylation, precore and HBV surface antigen (HBsAg) significantly decreased the abundance of the corresponding HBV RNAs [49-51]. In particular, the small HBsAg has been selected as a target for siRNAs because the major transcripts synthesized from this genomic region during HBV replication overlap and include the pregenomic RNA, which serves both as the template for reverse transcription and the synthesis of viral DNA and mRNA. In mice, injection of either HBsAg-specific siRNAs or plasmids encoding shRNAs, together with a plasmid encoding the entire

HBV genome, resulted in the decline in HBV serum marker levels [49,52]. Concomitantly, antiviral siRNA treatment greatly reduced the number of HBsAg- and core-positive hepatocytes. SiRNA treatment did not cause inflammatory infiltrate in liver tissue [49].

Targeting cellular factors involved in viral multiplication

The first cellular molecules to be involved in viral multiplication are receptors responsible for virus binding and entry into the cell. These receptors are particularly interesting targets for silencing, because the absence of such receptors blocks all subsequent steps of the viral cycle. Gaggar et al. [53] used CD46-specific siRNAs to demonstrate the role of CD46, a ubiquitously expressed complement regulatory protein, as a cellular attachment receptor for most group B adenoviruses. Similarly, the role of the ubiquitous glucose transporter GLUT-1 as a component of the receptor for the human T cell leukemia virus (HTLV) has been demonstrated by down modulating endogenous GLUT-1 expression with siRNAs specific for the 3' NC region of GLUT-1 mRNA [54]. Caveolin-1 knockdown by PTGS reduced human coronavirus 229E infection, because this virus binds to CD13 in rafts and enters the cell through caveolae [55].

SiRNAs encoded by Adenovirus vectors have been used to confirm the role of putative cellular cofactors for HCV, since viral replication in Huh-7 hepatoma cells was substantially blocked by silencing either La (a protein potentially involved in HCV internal initiation of translation), polypyrimidine tract binding protein (a protein interacting with both 5'- and 3'-NC regions), or subunit gamma of eukaryotic initiation factors 2B (a cofactor of HCV cap-independent translation) [56]. Similarly, targeting the human RNA helicase p68 by specific siRNAs caused a reduction in the transcription of negative strand HCV RNA [57].

Inhibition of viral dissemination within a host and inhibition of pathogenesis by siRNAs.

Dengue virus (DENV) serotypes 1-4 are transmitted to humans by the mosquitoes *Aedes aegypti* and *Aedes albopictus*. A Sindbis virus expression vector was used to study the role of mosquito genes in determining mosquito competence and virus dissemination. Sanchez-Vargas et al. [58] engineered a Sindbis virus genome such that it contains the coding region for early trypsin (TrypEarl) protein, a regulator of the proteolytic cascade in the midguts of blood-fed mosquitoes. The recombinant Sindbis virus transcribed a 500 bp region of the TrypEarl gene. TrypEarl-siRNAs were produced in mosquito midguts, leading to degradation of TrypEarl mRNA. Importantly, this affected dissemination of DENV-2 to tissue in the head. PTGS can thus be used to identify cellular genes involved in viral dissemination in mosquitoes.

A baculovirus, *Autographa californica* nucleopolyhedrosis virus, has caused severe economic losses in the silk industry over many years. Interfering with genes essential for baculovirus virulence, such as a major nucleocapsid gene or an early transcriptional activator, inhibits viral infections and prevents the death of over 95% of insects [59].

Foot-and-mouth disease virus (FMDV) is a picornavirus and the etiological agent of a devastating disease of cloven-hoofed animals, foot-and-mouth disease. W. Chen et al. [27] engineered plasmids encoding capsid protein VP1-specific siRNAs and injected them subcutaneously into the neck of suckling mice. This treatment rendered mice less susceptible to FMDV and promoted their survival. Interestingly, preadministration of a plasmid encoding the VP1 transcript contributed to the protection of animals. This result is in agreement with the observation that the effect of siRNA may be long-lasting if target mRNA is continually available [60].

The murine model has also been used to show that siRNAs specific for highly conserved regions of the nucleoprotein (NP) or acidic polymerase (PA) of influenza A virus

inhibit viral replication *in vivo* [26]. The intravenous and intranasal routes of inoculation were used successively. Virus-specific siRNAs reduced virus titers in the lung and protected mice against lethal challenge with highly pathogenic avian influenza A viruses of the H5 and H7 subtypes [26]. IFN could not have been responsible for the inhibition of virus replication, because H5 viruses are resistant to the antiviral effects of IFNs. In addition, Ge et al. [41] have shown that siRNAs given after influenza virus infection reduce virus replication in the lungs of mice, demonstrating a therapeutic effect of siRNAs.

SiRNAs and the recovery from virus-induced abnormal cell proliferation.

Over 90% of human cervical cancers harbor a human papillomavirus (HPV) genome and co-operative effects of the HPV E6 and E7 genes drive cell transformation. E6 and E7 interact with key tumor suppressors, p53 and the retinoblastoma protein pRb, respectively. Silencing the E6 gene in cervical carcinoma cells positive for HPV16 resulted in selective E6 mRNA degradation and p53 nuclear accumulation. Stabilization of p53 was accompanied by induction of the expression of a cell cycle control protein, p21 (a protein targeted by p53), and reduced cell growth, although no substantial G1 arrest was observed [61]. SiRNA specific for E6 did not induce significant cell death. In contrast, silencing the E7 gene induced selective loss of hyper-phosphorylated cellular pRb and caused the cells to round up and to undergo apoptosis. Therefore, in the case of HPV-positive human carcinoma cells, treatment with E7-specific siRNA leads to selective killing of the cancer cells [61].

Furthermore, in a squamous carcinoma model in New Zealand White rabbits infected with a cottontail rabbit papillomavirus, the role of specific cellular genes in cell invasion has been shown by PTGS [62].

Rous sarcoma virus (RSV) causes abnormal proliferation and tissue disorganization in chick embryos. A plasmid encoding a derivative of RSV modified for use as a retroviral

vector and either RSV Gag-specific siRNAs or a nonspecific siRNA control have been introduced into the neural tubes of chick embryos by electroporation two days after fertilization [24]. The effects were assessed three days later. All embryos electroporated with RSV plus control siRNAs were dead by this time, with abnormal misplaced proliferative cells, whereas those receiving RSV plus Gag-specific siRNAs were indistinguishable from uninfected embryos. Therefore, RSV-induced abnormal cell proliferation and tissue disorganization can be reversed by PTGS [24].

The limits of RNA interference

Although RNA interference is an extremely powerful tool, this new technology has several limits. RNA viruses show a high degree of sequence diversity between different genotypes; and there is rapid evolution of quasi-species, by mutation and recombination, and the generation of reassortant genomes in the case of segmented genomes. These are major problems for the development of siRNA-based gene therapies [33]. In addition, large sections of highly structured, untranslated regions of viral RNA genomes are resistant to PTGS [33]. A few siRNAs even enhance expression instead of having a silencing effect. Others have an off-target effect and therefore silence the wrong genes [6,63,64]. SiRNAs may also cross-react with targets of limited similarity [63]. Escape mutants have emerged after treatment of cells with a single virus-specific siRNA, in particular when cells are infected at high MOI [15,65,66]. The design of multiple siRNAs targeting different regions of the viral genome, in particular well-conserved regions, reduces the probability of generating escape mutants. In addition, some studies have shown that transfection with siRNAs results in IFN-mediated activation of the Jak-Stat pathway and general upregulation of IFN-stimulated genes [67,68]. The effect was mediated by dsRNA-dependent protein kinase, PKR, and depended on the concentration of siRNAs [68]. Bridge et al. [67] also noted that some shRNAs produced by a

lentiviral vector induced an IFN response whereas homologous chemically synthesized siRNAs did not. However, some synthetic siRNAs do induce an IFN response [68]. SiRNAs caused Toll-like receptor 3 to induce type 1 IFN and sequence-independent mRNA degradation in the course of acute herpes simplex virus type 1 infection of keratinocytes [69]. It is therefore recommended to use the lowest effective dose of siRNAs or siRNA-encoding vectors [67-69].

PTGS suppressor proteins have been identified in plant and animal viruses, although the molecular mechanisms of silencing inhibition are still poorly understood [70]. Flock house virus (FHV), which belongs to the *Nodaviridae* family, encodes a B2 protein exhibiting a potent silencing-suppression activity in transgenic plants [71]. B2 is essential for FHV accumulation in *Drosophila* cells. Li et al. [71] demonstrated that FHV triggers strong virus RNA silencing and that the same virus is equipped with an effective silencing suppressor. It has been suggested that dsRNA-binding proteins, such as the reovirus outer shell protein sigma 3, could suppress PTGS-mediated antiviral defenses [70]. Vaccinia virus and human influenza virus each encode an essential protein that suppresses the RNA silencing based-antiviral response in *Drosophila*. The respective suppressors, E3L and NS1, are dsRNA-binding proteins inhibiting the IFN-regulated innate antiviral response [72]. These results suggest that PTGS can play a role in nucleic acid-based antiviral immunity in mammalian cells [72].

Conclusions

Most of our knowledge about the molecular mechanisms of PTGS comes from studies in plants, *C. elegans* and drosophila. In mammals, the mechanisms and the tissue-specific characteristics of PTGS still need to be investigated in greater detail. The role of PTGS as a natural antiviral response in mammalian cells has yet to be confirmed, and the transmission of interference from one cell to another remains an interesting question that should be investigated. The identification of viral proteins as PTGS suppressors in mammalian cells has just started [72] and the various effects of viruses on PTGS in cells need to be studied. Nevertheless, PTGS is already extremely useful as a new approach in viral functional genomics and is already widely used to characterize the role of cellular proteins in the cycles of many viruses. The possibility of partial silencing allows the role of proteins required for cell viability to be studied.

For both preventive and therapeutic effects of siRNAs, it may be useful to reinforce some of the cellular pathways involved in PTGS. In animals, disease prevention by virus-specific siRNAs may be useful. In humans, the therapeutic properties of siRNAs will probably be developed before preventive applications. Today, there remain many problems concerning how to deliver the appropriate amounts of the desired siRNAs to the appropriate tissue, at the right time. These siRNAs, after testing for the absence of toxicity, will have to be stabilized, probably by chemical modifications, in order to obtain long-lasting effects. Despite these current limitations, the siRNA technology, targeting either viral or cellular genes, is very promising for the development of treatment for diseases for which neither vaccines nor efficient therapy are currently available.

Acknowledgements

The authors are very grateful to Laurent Blondel for his precious help with the figures.

This work was supported by grants from the Institut Pasteur.

siRNAs

+ Transfection agent

Cell

5'OH 3'OH
3'OH 5'OH

kinase

5'P 3'OH
3'OH 5'P

5'P 3'OH
3'OH

helicase

RISC (complex)

3'OH 5'P

RISC

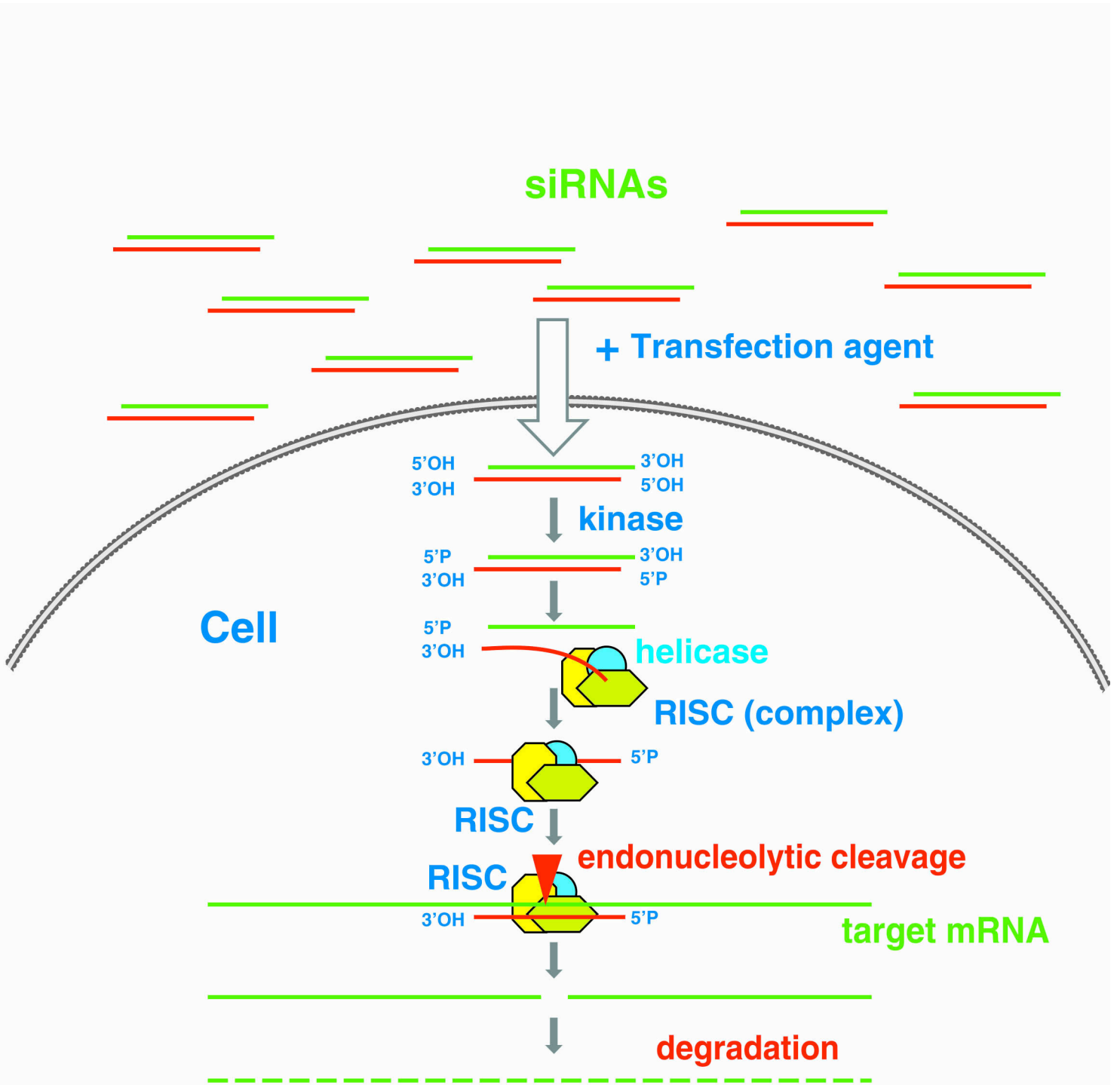
RISC

endonucleolytic cleavage

3'OH 5'P

target mRNA

degradation



Legend to Figure 1

A schematic model of gene silencing by synthetic siRNAs. Once transfected into cells, synthetic siRNAs are phosphorylated and a helicase, which may be associated with the RNA-induced silencing complex (RISC), leads to siRNA unwinding. The antisense strand of siRNA guides the complex towards the cognate mRNA. The target RNA is cleaved by an endoribonuclease in the RISC in a homology-dependent manner, resulting in mRNA degradation (see [3,4] and references in the text).

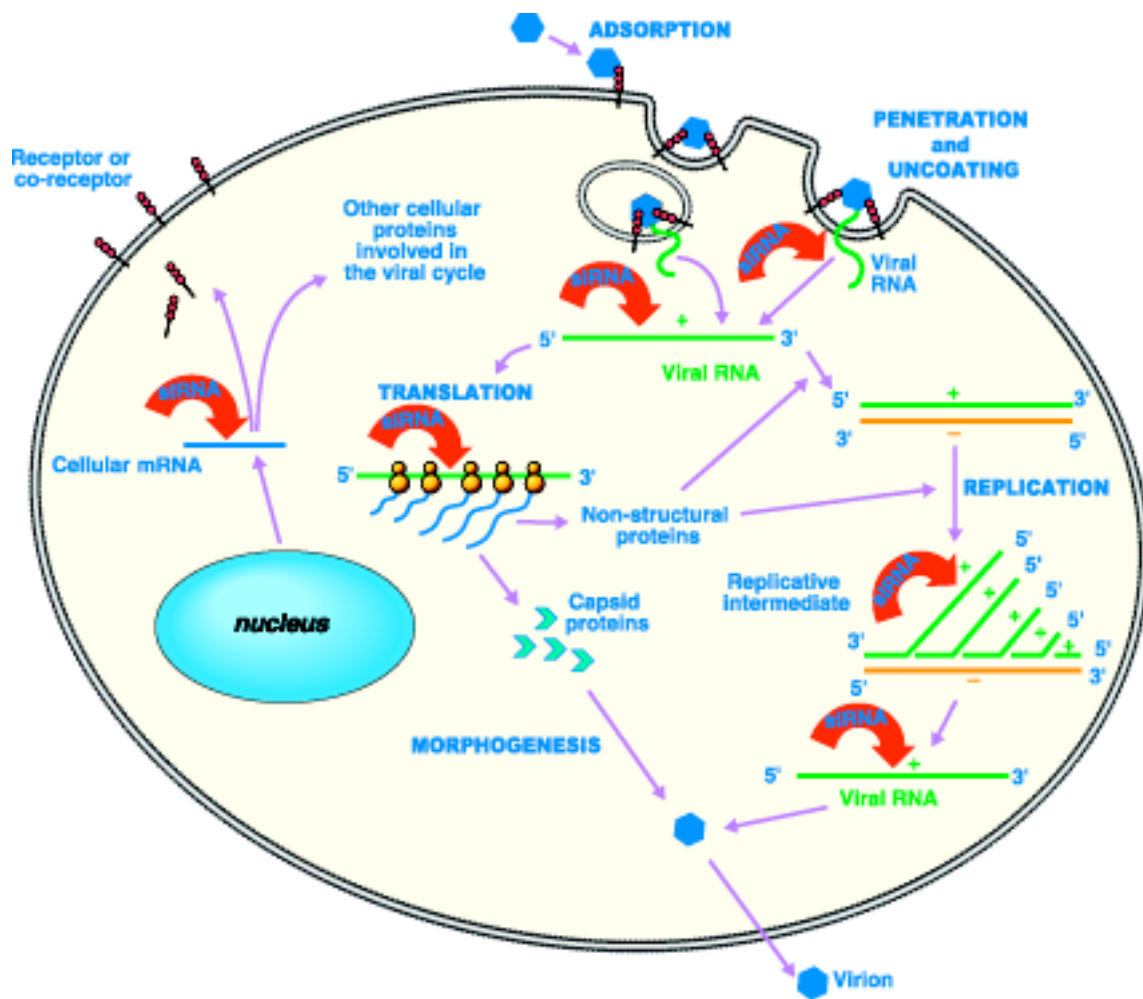


Figure 2

Legend to Figure 2

Schematic representation of the multiplication cycle of a RNA⁺ virus with potential siRNA targets. Silencing cellular mRNAs encoding proteins involved in viral multiplication is illustrated on the lefthand side of the figure, and silencing viral RNAs is illustrated on the righthand side of the figure.

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