Applications of RNA Interference in Mammalian Systems*

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Abstract

RNA interference (RNAi) can mediate the long- or short-term silencing of gene expression at the DNA, RNA, and/or protein level. Although several triggers of RNAi have been identified, the best characterized of these are small interfering RNAs (siRNAs), which can decrease gene expression through mRNA transcript cleavage, and endogenous microRNAs (miRNAs), which primarily inhibit protein translation. An improved understanding of RNAi has provided new, powerful tools for conducting functional studies in a gene-specific manner. In various applications, RNAi has been used to create model systems, to identify novel molecular targets, to study gene function in a genome-wide fashion, and to create new avenues for clinical therapeutics. Here, we review many of the ongoing applications of RNAi in mammalian and human systems, and discuss how advances in our knowledge of the RNAi machinery have enhanced the use of these technologies.

INTRODUCTION

RNA interference (RNAi): a gene-silencing mechanism induced by double-stranded RNA; can silence gene expression at both a transcriptional and post-transcriptional level

TGS: transcriptional gene silencing

RNA-induced silencing complex (RISC): central component of the RNAi pathway. RNAi effectors guide RISC to nucleic acids with at least partial complementarity

microRNAs (miRNAs): endogenous RNA species associated with RNAi that are generated through the processing of hairpin-containing transcripts

The awarding of the 2006 Nobel Prize in medicine for the discovery of RNA interference (RNAi) (**http://nobelprize.org/nobel_prizes/medicine/laureates/2006/**) clearly illustrates the importance of this field. First identified in *Caenorhabditis elegans* (31), and subsequently found to explain previous observations in plants and fungi (for reviews see 97, 141), RNAi is a gene-silencing mechanism that is induced by double-stranded RNA (dsRNA). Since its initial discovery, RNAi has been established in a number of additional organisms including *Drosophila melanogaster* (60, 90) and mammalian cells (15, 27). An increasingly diverse set of biological processes has been associated with RNAi. These processes include transcriptional gene silencing (TGS), antiviral responses, and, most relevant for this discussion, the post-transcriptional regulation of endogenous gene expression. Methods that exploit RNAi have been rapidly developed. Although primarily used for gene function analysis, ranging in scale from the individual gene to genome-wide, RNAi-based technologies have been used in a number of contexts including clinical applications.

The initial descriptions of RNAi focused on the post-transcriptional suppression of target genes mediated by the introduction of homologous dsRNA [over ≈100 nucleotides (nts)] into model organisms. Subsequently, these dsRNAs were found to be processed into smaller 21–23 nt dsRNAs, termed small interfering RNAs (siRNAs), with 3′ dinucleotide overhangs generated by the RNase III endoribonuclease Dicer (4, 28). siRNAs were found to be active independent of processing from larger dsRNAs (28). As the immune response precludes the use of long dsRNAs in mammalian cells, it was not until this discovery that RNAi could be identified in these systems (15, 27).

siRNAs direct the cleavage of targeted mRNAs. Cleavage is mediated by a single strand of the siRNA duplex, termed the guide strand, after incorporation into a ribonucleoprotein complex known as the RNA-induced silencing complex (RISC). RISC contains Argonaute proteins. This family of proteins is highly diverse, but all members are characterized by the presence of two domains, the Piwi-Argonaute-Zwille (PAZ) and PIWI domains (for review see 106). The PAZ domain specifically recognizes the characteristic 3′ termini of processed effectors and the PIWI domain adopts an RNase H-like structure that can catalyze the enzymatic cleavage of RNA. There are eight known Argonaute proteins in humans, but of these only Argonaute 2 (Ago2) has been found to generate cleavage-competent RISC (77). In addition to target cleavage, Ago2 is also responsible for guide strand selection. This occurs through the asymmetric unwinding of duplex RNAs, whereby the guide strand is preferentially retained within RISC and the other strand, termed the passenger strand, is degraded (85, 112).

Although the introduction of exogenous siRNAs results in the RISC-dependent cleavage of target transcripts, the documented occurrence of endogenous cleavage complexes is not common in mammalian cells. Rather, it is another species of small RNA, termed microRNAs (miRNAs), that uses the innate RNAi machinery. (For a detailed discussion and key references related to miRNAs, see the Mendell review in this volume.) miRNAs interact with transcripts possessing partial complementarity, primarily within target 3′ untranslated regions (UTRs). miRNAs were originally identified as a species of small RNA (≈22 nt) that regulates genes required for development in the nematode *C. elegans*. Known as small temporal RNAs, these were the first examples of a large number of small endogenous RNAs that can regulate gene expression (see **http://microrna.sanger.ac.uk/** for a database of all miRNAs). miRNAs are generated through the processing of genomically encoded primary miRNA transcripts (pri-miRNAs) by a multisubunit complex

that at its core consists of the RNase III endoribonuclease Drosha and, in mammalian cells, the DGCR8 protein. The processing of primary miRNA transcripts yields hairpin structures known as precursor miRNAs (pre-miRNAs). Following export to the cytoplasm via Exportin5 (Exp5), pre-miRNAs are processed by Dicer to produce mature miRNAs that incorporate into miRNA ribonucleoprotein complexes (miRNPs). These complexes are similar, if not identical, to RISC. Early studies suggested that mismatches between miRNAs and target transcripts resulted in translational repression without corresponding loss of mRNA. However, more recent studies have shown that miRNAs can induce reductions in target mRNA levels. Furthermore, miRNAs have been shown to colocalize with their target transcripts in sites of mRNA degradation known as cytoplasmic processing bodies, or P-bodies, where degradation through a process of cap removal followed by 5′–3′ exonuclease activity may occur. It has been estimated that miRNAs may regulate at least 30% of protein-encoding genes. Consequently, miRNAs are emerging as a fundamental regulatory unit in human biology. They have been linked to a number of important processes including development, differentiation, and even cancer. Understanding the role of miRNAs in human disease will be a major area of study in the coming years and may enable the development of new therapeutic approaches (see Sidebar: microRNAs).

Although originally thought to be restricted to post-transcriptional gene silencing within the cytoplasm of mammalian cells, RNAi has since been found to occur within the nucleus and to regulate a number of additional processes (**Figure 1**). For example, nuclear-localized RNAs can be degraded through RNAi (116). Furthermore, RNAi can induce TGS. Studies have shown that RNAi-mediated TGS can occur through DNA methylation and/or histone modification through the targeting of promoter regions (17, 58, 92, 137, 146). Others have found that TGS can occur independent of these DNA modifications (54). RNAi can also regulate selfish genetic elements. For example, endogenous siRNAs generated from within the 5′ UTR of the LINE-1 (L1) element can regulate L1 retrotransposons. Also consistent with a role for RNAi in the regulation of selfish genetic elements, *Dicer* knockout murine embryos exhibit an increased abundance of certain endogenous retroviruses (for review see 47).

In addition to miRNAs and siRNAs, other innate RNAi effectors have been identified. One class of these is the Piwi-interacting RNAs (piRNAs). piRNAs seem to be uniquely expressed in the mammalian germline, particularly in the testes (1, 34, 38, 145). The functional role of piRNAs is currently unclear, but a role in spermatogenesis is likely. Furthermore, a number of other small RNAs associated with RNAi have been identified in different species, including trans-activating siRNAs (tasiRNAs), studied in plants and nematodes, and small scan RNAs (ScnRNAs), found in *Tetrahymena* (for review see 62). To date, it

microRNAs

The analysis of microRNA (miRNA) expression profiles has indicated a relationship between miRNA expression and various biological processes including cancer (for review see 13). For example, the overexpression of hsa-miR-155 has been reported in Hodgkin's and some Burkitt's lymphomas (64, 65, 88) and is also associated with poor outcome for patients with lung cancer (152). Conversely, the downregulation of two genomically adjacent miRNAs, hsa-miR-15 and hsa-miR-16, has been reported in chronic lymphocytic leukemia (14). In addition to these types of correlations, a number of studies have identified the potential for miRNAs to act as oncogenes or tumor suppressors. For example, the expression of hsa-miR-155 can induce splegnomelema in transgenic mice (21). Additionally, the expression of miR-15 and miR-16 can induce apoptosis in leukemia cells (18). Notably, single-stranded oligoribonucleotides with 2′O-methyl modifications can inhibit the activity of complementary miRNAs (50). Thus, the use of these inhibitors has been suggested as a possible therapeutic approach.

Exp5: Exportin5 protein

UTR: untranslated region

RNAi effector: a small RNA that can direct RNAi against complementary targets (e.g., siRNAs or shRNAs)

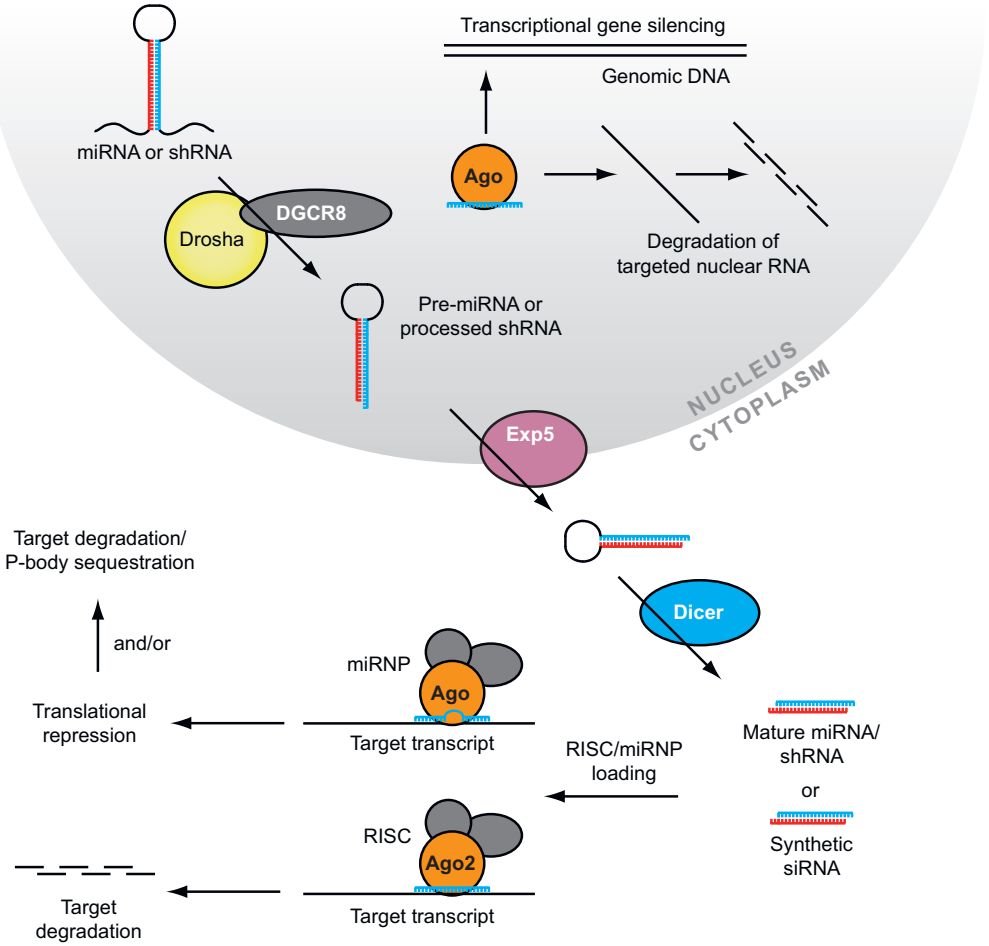

Figure 1

Simplified schematic of the RNAi pathway in mammalian cells. Only processes mentioned in the text are illustrated.

is unclear whether these types of small noncoding RNAs are also present in mammalian systems. However, it is apparent that RNAi-associated small RNAs can have an enormous effect on a variety of biological processes (see **Figure 1** for an overview of RNAi in mammalian cells).

INDUCING RNAi IN MAMMALIAN CELLS

In most cases, the aim of RNAi-based experiments is the sequence-dependent cleavage and reduction of protein-encoding mRNAs. Although most studies have focused on the RNAi analysis of these targets, any RNA species can be targeted (for example, noncoding RNA transcripts or viral RNAs). Only a limited number of mammalian cell types can tolerate RNAi induced by large, exogenous dsRNAs (e.g., embryonic stem cells). Thus, it is usually necessary to use one of two broad categories of RNAi effector molecules in mammalian systems. These include siRNA duplexes, formed through the annealing of two independent RNA strands, or single-stranded RNA molecules that contain a dsRNA domain, termed

short-hairpin RNAs (shRNAs). In both cases, RNAi effectors are designed to possess full complementarity with target transcripts, thereby resulting in their cleavage.

RNAi Effectors Used for Biological Analysis in Mammalian Cells

siRNAs can be generated through the annealing of synthetic oligonucleotides. Most synthetic siRNAs consist of 19 perfectly matched complementary ribonucleotides and 3′ dinucleotide overhangs that, for ease of synthesis, often consist of deoxyribonucleotides. Synthetic siRNAs are available from a number of commercial vendors. More rarely, siRNAs are generated by a number of other methods including in vitro transcription, plasmid-based tandem or convergent expression cassettes, polymerase chain reaction (PCR) or the endonuclease digestion of large dsRNAs that produce pools of siRNAs. The introduction of synthetic siRNAs into cultured mammalian cells usually uses standard physico-chemical transfection methods, such as those based on cationic lipids, cationic polymers, or electroporation. Empirical testing is required to determine the most efficacious transfection conditions for any given cell system. Once well-optimized, transfected siRNAs can yield a substantial decrease in the steady-state levels of target mRNAs for ~24–120 h.

As opposed to direct transfection, shRNAs are usually expressed from plasmids or viral-based expression vectors. shRNAs are designed to mimic miRNA precursors. Consequently, they are processed by the endogenous RNAi machinery and loaded into RISC. A number of different shRNA expression systems have been described. Variations include differences in promoter-terminator combinations, linker sequences, flanking sequences, duplex length, and regulatory elements that can be used for spatial and/or temporal-specific expression. Additionally, selection markers, used to generate stable cell lines, and unique sequence elements, used to identify active shRNAs among larger populations (discussed below), have been employed. As opposed to siRNAs, the stable expression of shRNAs allows for a nontransient reduction of targeted mRNAs. Thus, the choice of RNAi effector (siRNA or shRNA) depends on the question under investigation. No matter the choice, it is always important to include negative control effector molecules in any RNAi-based experiment (reasons discussed in detail below). These controls, which are commercially available, incorporate sequences with minimal complementarity to any endogenous transcript.

small interfering RNAs (siRNAs): annealed RNA duplexes of ~21 nucleotides in length that can direct RNAi; generally synthetic in origin

short-hairpin RNAs (shRNAs): hairpin-containing transcripts designed to mimic miRNA precursors; intracellularly processed shRNAs can direct RNAi

Maximizing Gene Silencing by RNAi

In addition to improving conditions for their cellular introduction and/or expression and subsequent processing, many studies have been directed toward maximizing activity as a function of effector sequence (61, 121). For example, an understanding of any bias related to guide strand selection has obvious implications for design. Analysis has revealed that the strand most easily unwound from its 5′ end is preferentially incorporated into RISC (61, 115). Thus, effector design incorporates such bias to encourage selection of intended guide strands. Studies have indicated other positional biases. For example, high thermodynamic stability is preferred between nucleotides 5–10 of the guide strand (61, 115). Furthermore, empirical comparisons between large sets of effective and ineffective siRNAs have led to the development of algorithms that assist in the generation of active siRNAs. These types of design tools are incorporated into the production of commercial siRNAs and are also publicly available. Sequences generated by these tools merely have an increased probability of mediating RNAi. Only experimentation will establish the activity of any given RNAi effector. Of note, an increasing number of validated sequences are available from commercial sources and are being characterized and collated by the scientific community

off-target effects: unintended effects arising through the application of RNAi; can occur in a sequence-dependent or -independent manner

(**http://www.ncbi.nlm.nih.gov/projects/genome/RNAi/**).

Considerations of the target are also important for maximizing RNAi. As RNAi effectors are designed according to reported reference sequences, any discrepancies between those and the actual target sequences within systems under study, for example as a consequence of single nucleotide polymorphisms (SNPs), may prevent efficient RNAi (84a). However, the influence of sequence discrepancies may be less than predicted owing to the fact that RISC can sometimes tolerate mismatches within targets, especially those distal from the cleavage site (26, 84). Despite possibly interfering with RNAi, sequence aberrations can potentially be used to selectively target mutated transcripts associated with disease. This approach has been applied in a number of contexts including the targeting of cancer-specific mutations, the targeting of a single-base mutation associated with the dominant genetic disorder spinocerebellar ataxia, and, most recently, for the silencing of mutant β-globin as an approach toward treating sickle cell anemia (9, 26, 89). In addition to potential sequence discrepancies, one should also ensure that RNAi effectors target all known transcript variants of genes under study (84a). Inevitably, even with an increased understanding of RNAi, effectors invariably exhibit a spectrum of activity. Thus, it may be prudent to obtain more than one effector against targets under investigation.

OFF-TARGET EFFECTS ASSOCIATED WITH RNAi

The ability of RNAi effectors to elicit specific downregulation of intended targets while minimizing or controlling for unintended effects, termed off-target effects, is critical for the meaningful application of RNAi. Off-target effects are known to arise from a variety of mechanisms, which include both sequence-independent and sequence-dependent processes. Sequence-independent effects, or nonspecific effects, generally involve those relating to transfection conditions (e.g., lipid transfection reagents), inhibition of endogenous miRNA activity, or stimulation of pathways associated with the immune response. Sequence-dependent effects primarily concern the unintentional silencing of targets sharing partial complementarity with RNAi effector molecules through miRNA-like interactions, but also include receptor-mediated immune stimulation through the recognition of certain nucleotide motifs. As discussed below, there are a number of approaches toward controlling for both types of off-target effects (summarized in **Table 1**).

Nonspecific Effects: Disruption of Endogenous RNAi

Nonspecific effects resulting from the inhibition of endogenous miRNA activity appear to depend on saturation of Exp5 (37, 154).

Table 1 List of off-target effects encountered in mammalian systems and steps to minimize their impact

Off-target effect	Type	Steps to mitigate
Saturation of the endogenous RNAi machinery	Sequence-independent	Use effectors at lowest possible concentration
		Use negative control effectors for comparison
Immune response	Sequence-independent	Use effectors at lowest possible concentration
Immune response	Sequence-dependent	Avoid known stimulatory motifs
		Use chemically modified effectors
		Use multiple effectors to confirm phenotypes
Silencing of unintended targets through partial complementarity	Sequence-dependent	Use effectors at lowest possible concentration
		Use multiple effectors to confirm phenotypes

For example, the shRNA-mediated inhibition of miRNA activity is mitigated by the overexpression of Exp5 (37, 154). Also consistent with Exp5 as a saturatable component of RNAi, its overexpression, but not the overexpression of other RNAi components, enhances the activity of both miRNAs and shRNAs (37, 154). As siRNAs do not require export from the nucleus, their activity would not be expected to depend on Exp5. However, some studies have found that siRNA-mediated RNAi is dependent on Exp5, where Exp5 prevents entry, and subsequent dilution, of siRNAs into non-nucleolar areas of the nucleus (102). This is still controversial, as other studies have found no relationship between Exp5 and siRNA activity (37, 154). At the very least, it is clear that the activity of endogenous miRNAs can be disrupted by the overexpression of shRNAs. The consequences of this not only manifest in cell culture, but also in vivo, as Grimm and colleagues (37) have shown that a high percentage of shRNAs can cause lethality in mice regardless of shRNA target, or even the presence of a target. Moreover, this toxicity correlated with high shRNA expression (37). Findings that RNAi effectors can saturate the endogenous machinery emphasize the importance of using RNAi effectors at the lowest possible effective concentrations. Additionally, the use of negative control siRNAs or shRNAs is paramount for the proper interpretation of results. In the case of shRNAs, it does not seem adequate to simply use an empty vector control, as this does not control for shRNA-mediated inhibition of the endogenous miRNA machinery.

Nonspecific Effects: The Immune Response

Despite early perceptions that siRNAs of less than 30 nts would avoid the immunostimulatory activity exhibited by larger RNA molecules (15, 27), it has since been shown that siRNAs can activate the immune response in a sequence-independent, concentration-dependent manner (57, 109, 114, 128). For example, 21-nt siRNAs have been shown to induce an interferon response in human glioblastoma T98G cells through a process dependent on the activation of the dsRNA-dependent protein kinase (PKR), and at least partially dependent on siRNA concentration (128). Similarly, both externally delivered siRNAs and shRNAs were found to induce an interferon response in HEK293 and HaCaT keratinocyte cell lines (57). Additional studies in HEK293 cells found this response to be primarily dependent on Toll-like receptor 3 (TLR3) (57). Importantly, the induction of an interferon response is cell-line dependent, with long siRNAs of 27 nt unable to activate a response in certain cell lines, including HeLa cells (114). The expression of shRNAs can also induce an interferon response (8). As with siRNAs, shRNA-mediated activation also appears to be concentration dependent (8). Thus, similar to saturation of the endogenous RNAi machinery, the use of lowest effective concentrations and negative control RNAi effectors are necessary to control for stimulation of interferon-type responses. Additionally, chemical modifications that help prevent the activation of PKR have been described (110).

Sequence-Dependent Effects: The Immune Response

siRNAs can also induce an immune response through sequence-dependent effects, particularly when it is part of a lipid or polycation complex in vivo. More specifically, certain nucleotide motifs, especially GU-rich sequences, can induce interferon-α (IFN-α), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), probably through activation of TLRs. For example, a subset of liposome-encapsulated siRNAs was found to induce a substantial, dose-dependent IFN-α response in mice (56). These siRNAs also stimulated an immune response in human peripheral blood mononuclear cells and isolated plasmacytoid dendritic cells. The stimulatory siRNAs were found to share

UGUGU motifs that were presumably recognized by endosomal TLR7 and/or TLR8 (56). Similarly, siRNAs were found to stimulate IFN-α production in human plasmacytoid dendritic cells through a GUCCUUCAA motif (48). In this case, experiments confirmed that stimulation was dependent on recognition by TLR7. Because nonimmune cells do not express detectable TLR7 or TLR8, sequence-dependent immune stimulation is not thought to influence experiments conducted in commonly used cell lines (83), but sequence-dependent stimulation is clearly an important issue regarding in vivo applications.

Sequence-Dependent Effects: Shared Complementarity Between Effectors and Nontargeted mRNAs

Although specific siRNA nucleotide motifs can activate an immune response, the primary source of sequence-dependent off-target effects originates from partial complementarity between RNAi effectors and off-target transcripts. Such interactions are similar to those exhibited by endogenous miRNAs, which usually share complementarity between nucleotides within their 5′ ends and regions within target 3′ UTRs (70). In fact, much like miRNA targets, off-targeted transcripts are enriched in those containing complementarity between their 3′ UTRs and hexamer (nts 2–7) and heptamer (nts 2–8) sequences within 5′ ends of RNAi effectors (5, 52). Some studies have found these effects to be nontitratable, with dose responses mirroring that of on-target transcripts (51). Others have found these effects to be concentration-dependent, whereby the use of low siRNA concentrations can significantly mitigate off-target interactions (122). Importantly, most detailed studies of off-target effects are conducted using gene expression analysis. However, since miRNAs can impede translation in a manner disproportionate with alterations in target mRNA levels (24, 25), the magnitude of off-target effects may be underestimated.

Sequence-dependent off-target effects can have functional consequences. For example, different siRNAs targeting the same gene can exhibit varying effects on the mRNA and protein levels of key cellular genes, independent of on-target silencing (120). Accordingly, a high percentage of siRNAs can induce a toxic phenotype. For example, 51 of 176 randomly selected siRNAs directed against either firefly luciferase or human *DBI* reduced the viability of HeLa cells by more than 25%, a trend that was reproducible in different cell lines (30). From a practical perspective, off-target effects can have a profound effect on experimental results. For example, Lin and colleagues determined that the top three "hits" from a siRNA-based screen for targets affecting the hypoxia-related HIF-1 pathway resulted from off-target effects (76). For two of these three "hits," activity could be traced to interactions within the 3′ UTR of *HIF-1A* itself. Of note, off-target effects not only affect experiments conducted in mammalian systems, but can also influence studies in *Drosophila* (68, 81).

There are a number of ways to control for, and help minimize, sequence-dependent off-target effects. Many of these relate to siRNA design features. For example, the use of asymmetric design, which helps to minimize the loading of passenger strands into RISC, thereby reducing associated off-target effects, and the use of siRNAs designed to avoid homology with untargeted transcripts. Both of these considerations are typically incorporated into the design of commercially available siRNAs. Increased stringency may be gained through the development of new algorithms that include emphasis on avoiding complementarity between siRNAs and untargeted 3′ UTRs (5). Chemical modifications that reduce sequence-dependent off-target effects have also been described. For example, the incorporation of 2′-O-methyl groups within the first two 5′ nucleotides of siRNA passenger strands reduces passenger strand-mediated activity (52). Similarly, a 2′-O-methyl ribosyl substitution at position 2 of the guide strand can significantly

reduce sequence-dependent off-target effects (52). Modified siRNAs exhibiting reduced off-target effects are commercially available. As with sequence-independent effects, experimental conditions should be optimized to use the lowest effective dose of the RNAi effector.

Despite all of these considerations, the occurrence of sequence-dependent off-target effects may be unavoidable. Consequently, efforts should be made to help validate hits. All RNAi-derived phenotypes should be confirmed with additional RNAi effectors against the same target. Moreover, the downregulation of target mRNA and protein levels should be characterized and correlated with the observed effects. For example, the inactivity of a follow-up siRNA does not necessarily imply that the activity of the first resulted from off-target effects, especially if the second siRNA is unable to downregulate target levels. Conversely, a phenotype induced by only a fraction of siRNAs directed against the same target, despite equivalent silencing by all siRNAs, would be suspicious. Overall, it is difficult to prescribe the number of independent RNAi effectors necessary for target validation, but it would certainly require at least two. No matter how many independent RNAi effectors are tested, it could be possible that observed phenotypes result from cooperative effects between target-specific downregulation and nonspecific effects. Even a rescue experiment using a target construct resistant to RNAi could not control for such scenarios. Thus, confirmation of phenotypes under different experimental conditions (e.g., the use of a different lipid reagent or the use of an siRNA to confirm an shRNA-derived phenotype) may help to eliminate some of these possibilities. Additionally, RNAi-independent methods, such as the chemical inhibition of identified targets, should be used to corroborate phenotypes where possible.

STUDIES USING RNAi

The types of studies using siRNAs or shRNAs have been highly diverse and are being performed on an increasingly routine basis. Most reports describe the knockdown of one or a limited number of genes directly relevant to the biological question under investigation. Other, slightly broader studies have used RNAi to identify causal genes within disease-associated genomic regions (74). Analogously, RNAi is being used to validate statistical relationships between gene expression and phenotypes and to investigate compound modes of action. For example, RNAi has been used to identify both biomarkers associated with and genes responsible for compound activity (78, 79). However, in contrast to these focused applications, it is the development of large-scale RNAi analysis that has the greatest potential to impact the discovery of novel gene function.

RNAi-Based Screens

The generation of whole genome sequences for human and an increasing number of model organisms has presented biologists with an extremely valuable resource. To use this information fully, it is essential to develop complementary analytical approaches that can be performed on a similar scale. Studies of DNA copy number by comparative genomic hybridization, mRNA expression by microarray, and the profiling of the proteome by various methods have all been adapted for large-scale analysis. Now the use of RNAi-based methods is enabling corresponding functional analysis to be conducted on a similar scale. Genome-wide RNAi analysis was first developed for the model organisms *C. elegans* (see **http://www.wormbase.org/db/searches/rnai_search** to search for *C. elegans* RNAi phenotypes) and *Drosophila* (see **http://flyrnai.org/cgi-bin/RNAi_screens.pl** for details of a number of large-scale screens). Recently, analogous RNAi screens were described in human cells.

Several different strategies for large-scale RNAi-based screening in mammalian cells have been developed (see **Figure 2** for an overview). Perhaps the most straightforward

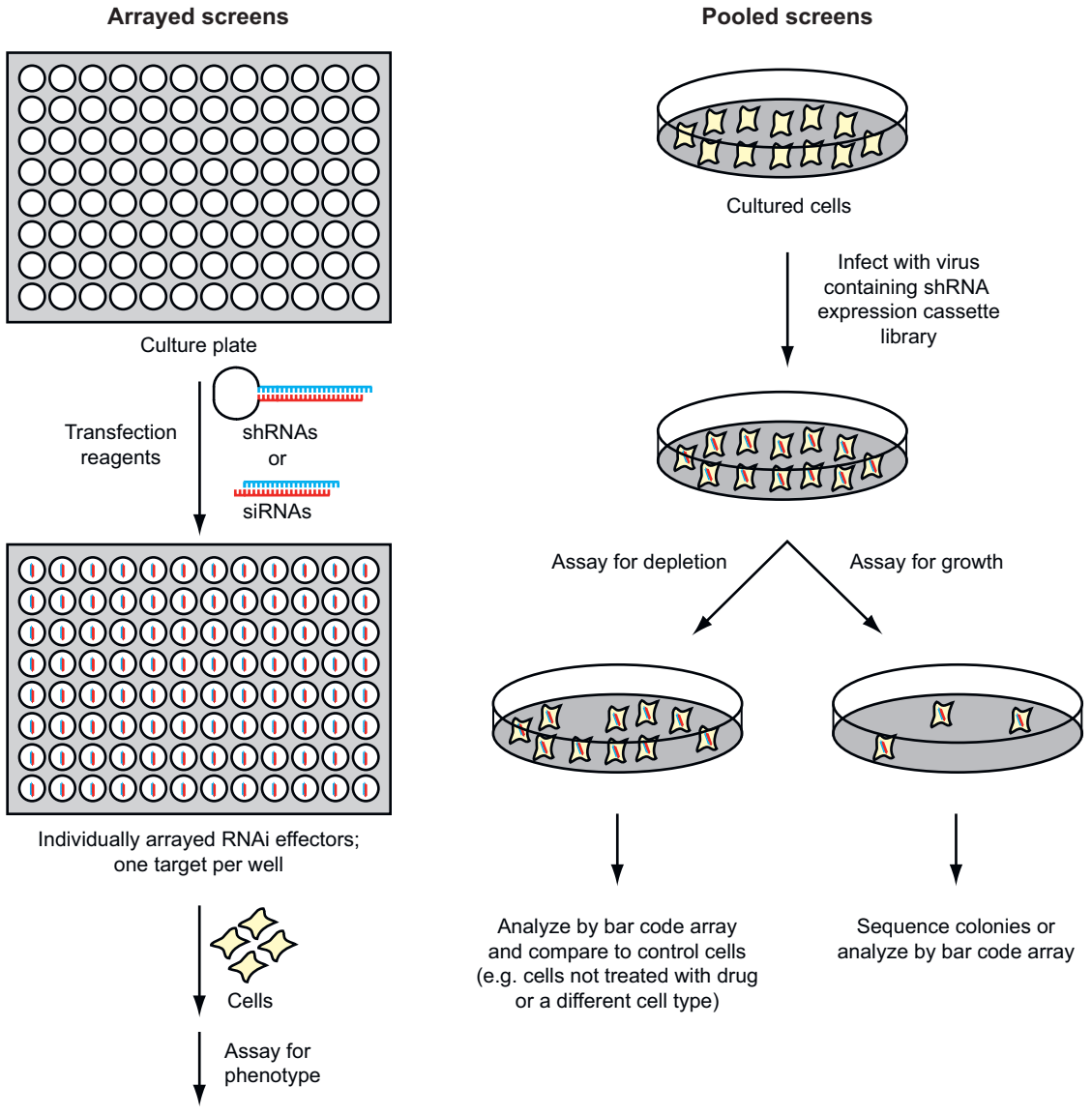

Figure 2

The different formats for conducting RNAi-based screens. Arrayed screens (*left*) are usually conducted in standard tissue culture plates where one gene target is assayed per well. This type of format is used for both small interfering RNA (siRNA)- and short hairpin RNA (shRNA)-based screens. As illustrated, RNAi effectors can be precomplexed with transfection reagents followed by the addition of cells. In most screens, effectors are evaluated in at least duplicate and usually a number of sequence-independent effectors are evaluated per target. Pooled screens (*right*) are usually conducted by infecting cells with an shRNA library in a "one pot" format. Active shRNAs are identified through sequence analysis or by bar code array.

involves the use of arrayed synthetic siRNA libraries. These libraries are available from a number of commercial vendors and in a variety of formats, ranging from a genome-wide scale to smaller customized subsets. A number of studies using these types of resources have been described. Some of these involve the elegant integration of siRNA screens with image-based high content analysis. For example, a screen of siRNAs directed against the human kinome identified regulators of clathrin and caveolae-mediated endocytosis by imaging the internalization of vesicular stomatis virus and simian virus 40, respectively (107). Similarly, a more focused screen, restricted to known endocytosis-related genes, identified those associated with endocytosis of the dopamine transporter (130). Recently, a fully automated process was used for screening siRNAs with a time-lapse imaging system in human cells (98).

Synthetic siRNA-based screens are also used to identify novel molecular targets, both in the context of stand-alone targets and those augmenting the activity of existing chemotherapeutics. For example, a screen of ~5000 genes found several associated with migration in highly mobile SKOV-3 cells (20). The activity of one identified target, MAP4K4, was mediated through the c-Jun N-terminal kinase, leading to the rational use of a c-Jun N-terminal kinase small molecule inhibitor to mitigate SKOV-3 motility (20). Additionally, a screen of human kinases and phosphatases identified those contributing to the circumvention of apoptosis in cancer cells (82). Several novel regulators were identified, including PINK1 kinase, whose inhibition was shown to sensitize HeLa and BT474 cells to taxol (82). Analogously, a screen identified targets that enhanced TRAIL-induced apoptosis in HeLa cells (2). This type of strategy provides an important avenue toward identifying rational drug combinations, which may result in potent therapies that avoid high doses, and associated side effects, of any one agent.

siRNA-based screens are usually conducted in standard plate-based formats (e.g., 96 or 384 well plates). However, the potential adaptation of RNAi screening to a microarray format has been explored. Several "proof of principle studies" have been conducted, whereby small RNAs are precomplexed with a carrier and arrayed on slides to enable the localized transfection of overlaid cells (29, 95, 127). Standard image-based systems can be used to assess resulting phenotypes. One potential advantage of this approach is that it may facilitate the generation of a more standardized format for use by the scientific community, thereby enabling more extensive cross-comparison of data.

Another major approach to large-scale RNAi-based screens is the use of shRNA libraries. A number of these systems have been described (3, 91, 99, 104, 126). Generally, these systems incorporate shRNAs into selectable viral vector plasmid backbones, with expression driven by RNA polymerase III promoters. Both retroviral and lentiviral systems have been described. Lentiviral systems are advantageous for certain applications, as they are able to infect primary and nondividing cells. Most shRNAs contained within these constructs are based on hairpin structures intended to mimic pre-miRNAs. Furthermore, they incorporate many of the same design rules as those used for synthetic siRNAs. Recently, a library based on the mimicry of primary, rather than precursor, miRNAs, was described (126). Such shRNAs generate upward of 12 times more mature small RNAs than those based on the mimicry of pre-miRNAs (126).

shRNA libraries have been screened in both arrayed and pooled formats (**Figure 2**). Inherently, a pooled format has a significant throughput advantage. However, pooling requires the ability to subsequently identify active shRNAs within treated cell populations. In some cases, sequence analysis can be used. This is especially possible when screening for selectable phenotypes that yield a growth advantage, resulting in the generation

bar code: the shRNA sequence or a unique sequence incorporated into shRNA expression cassettes that is used to identify individual shRNAs within pooled screens

of positive clones. For example, a screen using a system engineered to exhibit innate p53-dependent growth arrest identified five novel inhibitors of this process (3). Similarly, novel tumor suppressors were identified using shRNAs that transformed human primary cells (66). Despite the successful use of this strategy, there are a number of limitations. For example, using this type of approach it is difficult to screen for shRNAs that inhibit growth, thereby leading to the depletion of active shRNAs from the larger, inactive population. Furthermore, even in systems engineered to assay for growth advantages, high hit rates may make sequencing-based identification impractical. To remedy these issues, many shRNA expression systems employ an identification strategy based on the use of bar codes. Bar codes are unique nucleotide sequences that are used to help identify enriched or depleted shRNAs within treated cell populations. Bar codes can be assayed by DNA microarray analysis. Different bar code strategies have been employed. In one approach, the shRNA sequences are used as bar code identifiers (3). In a different approach, unique 60-nt bar codes are incorporated into shRNA expression cassettes (99, 104, 126). Several studies have demonstrated the utility of bar codes. In an elegant example, this strategy was used to identify molecular targets unique to a subset of diffuse large B-cell lymphoma (99). This was accomplished by using an inducible shRNA expression system, whereby bar code analysis of an induced versus noninduced cell population enabled the identification of shRNAs exclusively depleted from the induced set. As with siRNA-based screens, bar code screens can be used to probe the mode of action of anticancer agents. For example, a bar code strategy found that DNA-damage induced signaling enhances the activity of a small molecule MDM2 inhibitor, suggesting that optimal efficacy may be observed in cells with wild-type p53 and activated DNA-damage signaling (10). The successful application of pooled shRNA screens is promising, as it may make large-scale RNAi screens more accessible to laboratories lacking the extensive liquid-handling capabilities required for large arrayed screens.

In addition to synthetic siRNA and shRNA libraries, the generation of RNAi libraries from cDNAs has also been described. For example, *Escherichia coli* RNase III has been used to generate siRNAs from cDNA-derived dsRNA (63, 153). The screening of a library prepared by this strategy was able to identify novel regulators of cell division in HeLa cells (63). In another cDNA-based approach, expression systems are produced through a process partially dependent on the use of *Mme*1 restriction enzyme (80, 123, 125). This enzyme cleaves 20 nt from its recognition site, making it very amenable to the generation of siRNAs. As all of these systems are based on random generation from cDNAs, the generated effectors do not incorporate rational design features, possibly resulting in a relatively higher frequency of deleterious events, such as off-target effects. Despite the development of cDNA-based strategies, the uses of both siRNA and shRNA libraries are clearly the more abundant methodologies employed in large-scale screens.

Regardless of strategy, a key promise of large-scale RNAi functional analysis is its potential impact on molecular target discovery and drug development. This is well illustrated by an shRNA-based screen of deubiquitinating enzymes that identified cylindromatosis (CYLD) as a novel suppressor of NF-κB activation (11). Inactivating mutations in CYLD are linked to a rare genetic disorder known as familial cylindromatosis, which predisposes individuals to the development of certain skin tumors. As the activation of NF-κB is antiapoptotic, it was hypothesized that loss of CYLD in cylindromatosis results in aberrant growth due to inhibition of apoptosis. Consequently, the inhibition of NF-κB was hypothesized as a potential treatment for this disorder (11). The clinical impact of this finding was quickly realized with

the performance of a trial assessing the topical application of the NF-κB inhibitor salicylic acid (103). Of 12 treated lesions, 2 lesions showed complete remission whereas another 8 exhibited some response to treatment (103). Future RNAi-based studies will hopefully be able to link more diseases with existing therapeutics.

THE IN VIVO APPLICATION OF RNAi-BASED TECHNOLOGIES

RNAi has enormous potential for the treatment of many genetic and acquired diseases. For example, RNAi could potentially be used to reduce the levels of toxic gain-of-function proteins, trigger cytotoxicity within tumors, or block viral replication. The use of RNAi-based therapeutics is especially appealing as RNAi can be used to modulate the expression of proteins not normally accessible by more traditional pharmaceutical approaches. For example, nondruggable targets lacking ligand-binding domains or proteins sharing high degrees of structural homology that are difficult to target as individuals are all accessible by RNAi.

The in vivo application of RNAi was described within a year of the first cell culture experiments, with reports describing the transient inhibition of transgenes within the livers of mice. This was accomplished through high-pressure tail vein injection of both siRNAs and shRNAs (71, 86). Subsequent in vivo studies have focused on the improved delivery and efficacy of RNAi effectors. These efforts have used the experience gained through two decades of developing ribozyme and antisense-based therapeutics and the gene therapy field as a whole. Currently, most in vivo studies using synthetic siRNAs use lipid-based carriers with or without modification of the siRNA itself, whereas most shRNA-based studies employ the standard viral vector expression systems used in traditional gene therapy. As evidenced by ongoing clinical trials, significant progress has been made in the field of in vivo RNAi (for an overview of RNAi therapeutic strategies see **Figure 3**).

In Vivo Application of Synthetic siRNAs

The in vivo delivery of synthetic siRNAs must account for the need to ensure resistance to exonuclease digestion, the maintenance of duplex stability, good pharmacokinetics, and the minimization of nonspecific immunological responses. Accordingly, a number of siRNA chemical modifications that address these issues have been examined. Many of these modifications are analogous to those incorporated in RNase H-dependent antisense oligonucleotides. A common modification to improve stability is the use of a partial phosphorothioate backbone, particularly within the 3′ overhangs of both siRNA strands. Furthermore, the inclusion of 2′-O-methyl dinucleotides at the 3′ end of the antisense strand has also been shown to improve stability. As mentioned, avoiding immune stimulation is also critical. The selection of sequences that avoid GU-rich sequences and/or modification with 2′-O-Me nucleotides or locked nucleic acids (LNAs) have all been shown to inhibit stimulation of the immune system without concomitant loss of efficacy (48, 55). Chemical modifications have also been engineered to improve cellular uptake. For example, cholesterol-conjugated siRNAs, corresponding to the *ApoB* gene, have been delivered into the livers of mice as a potential strategy for the treatment of familial cholesterolemia and, possibly, for the broader treatment of atherosclerosis (131). These conjugates were found to induce a significant decrease in both liver *ApoB* mRNA and plasma ApoB protein levels, as well as downstream lipoprotein and cholesterol levels. These effects were much greater than those observed with nonconjugated analogs. Unfortunately, the quantity of material necessary for efficient silencing was incompatible with scale-up to larger preclinical models, thus follow-up studies in nonhuman primates

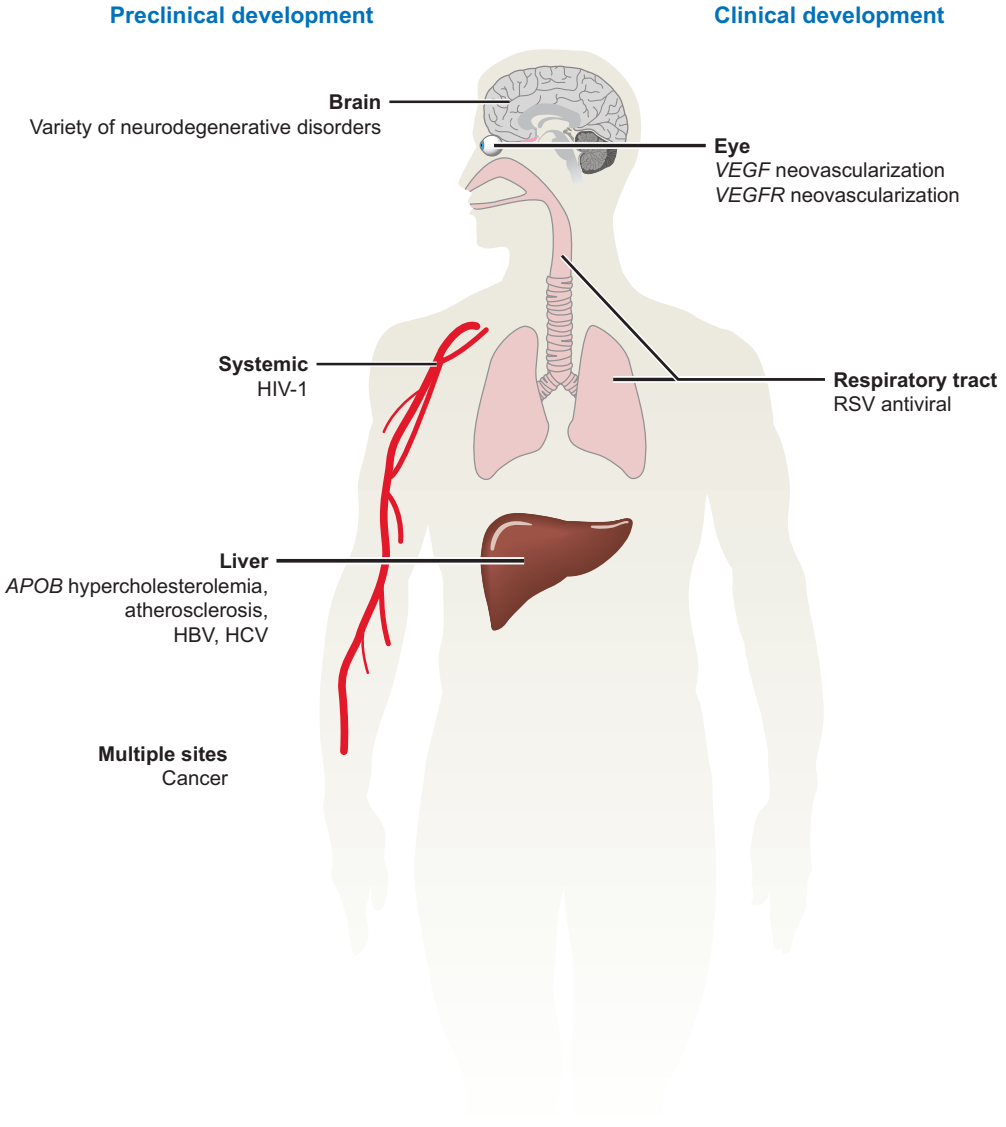

Figure 3

An illustration of the different in vivo applications of RNAi. A number of disorders have been targeted through RNAi in preclinical models (*left*). Many issues regarding the in vivo application of RNAi are analogous to those previously encountered in the field of gene therapy. Despite these complications, a number of RNAi-based therapeutics are currently in clinical development (*right*).

used a different delivery strategy (liposomal carriers, discussed below) (158).

In addition to directly modifying siRNAs for improved characteristics, carrier molecules also have the potential to protect siRNAs from the extracellular environment and improve intracellular delivery. A wide variety of polymer- or lipid-based delivery systems have been described. For example, cationic polyethylenimines have been

used for siRNA transfection in vivo, including delivery to lung and xenografts following subcutaneous, intraperitoneal, and intrathecal administration (39, 136). A large number of different liposome-based carriers have also been developed for the in vivo delivery of siRNAs (for review see 73). One such system that has been relatively well characterized uses lipid-polyethylene glycol (PEG) mixtures to encapsulate siRNAs. This delivery system has been used for the systemic delivery of *APOB*-targeted siRNAs into the livers of nonhuman primates, causing a significant reduction in both *APOB* mRNA and protein levels. Furthermore, a relatively sustained (11-day) reduction in low-density lipoprotein was observed in animals receiving the highest dose of siRNA lipid (2.5 mg/kg). Similarly, this lipid-encapsulated siRNA system has been used in studies directed toward inhibiting viral infections (33, 94).

In Vivo Application of shRNAs

The first studies applying shRNAs in vivo used plasmid DNA (71); however, most subsequent studies have focused on the use of viral vectors. The choice of viral delivery system usually depends on the cell type under investigation and on the need for short- or long-term shRNA expression. For example, adenoviral (AV) and herpes simplex viral vector systems have been primarily used for short-term expression, while adeno-associated viral (AAV) vectors and the integrating viral vector systems based on retroviruses (RVs) and lentiviruses (LVs) have usually been used for long-term expression or for applications in nondividing cells.

An important adaptation of RNAi has come from the ability to stably express shRNAs in blastocytes or embryonic stem cells, from which transgenic animals can be generated. Initial "proof of concept" experiments used shRNAs to target overexpressed marker genes (e.g., green fluorescence protein) in transgenic animals. These studies used either direct injection (41) or lentiviral transduction of early embryos (41, 138). Subsequent studies have demonstrated the feasibility of targeting endogenous genes within embryonic stem (ES) cells (16, 69, 118). These models broadly mimic the phenotype of traditional knockout mice. Consequently, the constitutive expression of an shRNA for the generation of an RNAi-based transgenic is only compatible with genes that do not compromise animal viability. To circumvent embryonic lethality, shRNA-based conditional expression systems have been developed. These include Cre-Lox-based systems whereby the shRNA is flanked by LoxP sites that prevent shRNA expression (32, 142). Tissue-specific or temporal-specific shRNA expression can then be achieved by crossing shRNA transgenic mice with *Cre* recombinase expressing mice (101, 142). Although Cre-lox RNAi-based systems are irreversible, reversible expression, predominantly using doxycycline-based control systems, has been described (133, 149). RNAi transgenics have also been used in animals not normally amenable to traditional homologous recombination techniques, including rat (22) and goat. In the case of goat, a RNAi transgenic was generated through somatic cell nuclear transfer from a LV-transduced goat fibroblast stably expressing an shRNA corresponding to the prion protein (35). While the development of RNAi transgenics was initially hailed with great excitement, its broader use has not been adapted as quickly as may have been anticipated. This may be due to difficulties in obtaining efficient lentiviral transfection of embryos or ES cells, difficulties in generating ES clones that stably express shRNAs or problems associated with variations in knockdown efficiency. The recent adaptation of the more conventional pronuclear injection procedure may enable wider use of RNAi in the development of transgenics (108).

shRNAs have also been used in xenograft tumor models, particularly in mice. One of the first examples of this was targeting an activated mutant of *K-RAS* found in the pancreatic carcinoma cell line CAPAN-1 using

a mutant-specific shRNA expressed from a retroviral vector (9). In contrast to control cells, the *KRAS*-targeted cells failed to form tumors in athymic mice, demonstrating the ability of RNAi-mediated silencing to suppress tumor formation in vivo. Another interesting example involves targeting the tumor suppressor *TP53* in mouse *Eμ-Myc* hematopoietic stem cells. By using shRNAs that mediated different degrees of *TP53* message reduction, the percentage of mice developing lymphoma could be varied as a function of TP53 protein levels (42). More recent variants of this method include the use of an inducible expression system that can be activated upon xenograft tumor formation, potentially generating a better clinical model for the identification and validation of anticancer molecular targets (59, 72).

The Development of RNAi-Based Therapeutics: Organ and Disease-Based Studies

The eye and neovascularization. The first clinical trial of an RNAi effector, a synthetic siRNA, was initiated just three years after the identification of RNAi in mammalian cells. Initially tested in mouse (113), the aim of these trials is to decrease expression of the vascular endothelial growth factor (VEGF) in order to suppress abnormal blood vessel development in the eyes of patients with age-related macular degeneration (AMD) and, more recently, diabetic retinopathy (**http://www.acuitypharma.com/**). A similar clinical trial in patients with AMD is being conducted with a siRNA targeting the VEGF receptor (**http://www.sirna.com/**).

The brain and neurological disorders. Using traditional transgenic approaches, it has been difficult to generate models of neurological disorders, including Parkinson's disease (PD). Thus, RNAi has been widely pursued as a new approach for developing models of neurological disorders. Due to the need for long-term suppression of gene expression and the need to treat nondividing cells, most brain-based studies have focused on the use of shRNA expression vectors derived from AAVs (44, 45, 117, 150) or LVs (36, 111). However, adenoviral systems (151), herpes-simplex systems (46), and synthetic siRNAs have also been used (134, 135). Examples of using RNAi to generate neurological or behavioral models include the introduction of an AAV shRNA against tyrosine hydroxylase into mid-brain dopamine neurons as a model for PD, as well as the AAV expression of an shRNA corresponding to the Leptin receptor as a model of feeding behavior (44, 45).

RNAi has also been explored for the potential treatment of neurodegenerative disease, particularly those associated with a dominant genetic inheritance. Many neurodegenerative disorders are associated with the expression of an aberrant protein that may be inappropriately aggregated, deposited, sequestrated, or mislocalized. Numerous groups have hypothesized that the induction of RNAi directed against these proteins may modulate disease progression. Associated studies have included the use of shRNAs to target the Huntingtin protein of Huntington's disease in mouse models (40, 117), the use of *DYT1* for the treatment of dystonia (36), and the targeting of *ataxin-1* to treat spinocerebellar ataxia (150). In addition, studies targeting the superoxide dismutase gene have been conducted in models of amyotropic lateral sclerosis (111).

The liver, metabolic disorders, and hepatitis (nonviral and viral). A number of RNAi-based strategies have been proposed to treat various liver diseases, including inherited metabolic diseases such as hypercholesterolemia (131, 158). However, most liver studies have focused on using RNAi to prevent hepatitis. One of the first of these studies investigated the feasibility of preventing liver damage caused by Fas-mediated apoptosis in a mouse model of acute hepatitis (129). Using a synthetic siRNA against *FAS*, which was introduced by hydrodynamic tail vein injection, a significant reduction in liver cytotoxicity and

substantial effects on liver pathology and survival were seen as compared with controls. In a similar study, the RNAi knockdown of *Caspase 8* was used to prevent acute liver failure (155). However, the main focus of RNAi liver studies has been the development of anti-Hepatitis B virus (HBV) or anti-Hepatitis C virus (HCV) strategies. Antiviral strategies using RNAi-based approaches fall into three broad categories: those targeting (*a*) a host gene required for viral infection (e.g., a cell surface receptor), (*b*) the viral genome itself, or (*c*) a viral transcript encoding a protein essential for virus replication. One initial study regarding HBV involved plasmids expressing shRNAs against pregenomic viral RNA and in some cases viral mRNAs. Using a plasmid DNA-based replicating HBV model system, this study showed that HBV-targeting shRNAs decreased HBV DNA replication and the expression of two viral proteins (87). Chemically modified synthetic siRNAs encapsulated in liposomes have also been used to target HBV. As with the shRNA-based system, siRNAs were shown to reduce both HBV DNA and HBV protein levels in a similar HBV model system (94). Importantly, the use of an adenoviral vector expressing HBV shRNAs in an HBV-transgenic mouse model suggests that RNAi can substantially reduce HBV gene expression and replication in an established HBV infection (140). Unfortunately, no robust in vivo model of HCV infection exists, but experiments in cell culture have shown that both siRNAs and shRNAs targeting either the HCV genome or different viral transcripts can significantly reduce viral replicon replication (67, 143, 148).

Other antiviral RNAi therapies. HIV-1 has been another important target for the development of RNAi-based therapeutics. A number of different anti-HIV strategies have been explored. These include targeting HIV-1 cell surface receptors (for example, CD4) as well as strategies that block viral integration or replication. For example, RNAi has been directed against the HIV-1 *gag* gene that encodes the major structural proteins of HIV-1 (100). Other targets have included the HIV-1 long terminal repeat, HIV-1 accessory proteins, and the regulatory proteins *tat* and *rev* (19, 53). Current studies are addressing the need to obtain long-term expression of anti-HIV-1 RNAi effectors (for review see 93).

Other antiviral RNAi studies have focused on using RNAi against the negative-RNA strand virus respiratory syncytial virus (RSV), which has a significant impact on children and the elderly. A number of promising anti-RSV strategies have been described. For example, the intranasal administration of a synthetic siRNA directed against RSV reduced viral titer in lung when administered before or at the time of viral infection. A less profound effect was observed when administered several days after infection (6). Additionally, an shRNA corresponding to a different RSV mRNA was also able to decrease viral titer in lung. However, as with the siRNA, minimal activity was seen when the RNAi effector was administered post-infection (157). Notably, a siRNA-based clinical trial targeting RSV has been initiated (**http://www.alnylam.com/**).

In addition to HIV-1 and RSV, RNAi-based therapeutics have been developed for several other viruses including Ebola (33), Herpes simplex virus 2 (105), and SARS coronavirus (75). No matter the target, an important consideration for any RNAi-based antiviral is sequence variations between different strains or isolates of the same virus. Additionally, viruses have the potential to generate RNAi-resistant mutations. For example, the long-term culturing of HIV-1 in cell lines expressing an anti-HIV-1 shRNA can result in high levels of sequence alterations (mutations and deletions) that render the virus resistant (7, 23, 147). Using multiple shRNAs targeting independent sequences may help to mitigate this effect, but sequence adaptation/variation represents a significant hurdle in the use of antiviral RNAi therapeutics.

Cancer. There are many events in cancer that could be directly targeted by RNAi-based

therapeutics. Especially amenable to RNAi are those cancers driven by a single genetic factor—for example, the oncogenic fusion transcript EWS-FLI1 that is associated with Ewing's sarcoma. Using a nonviral delivery method based on transferrin receptor targeting, an siRNA corresponding to the *EWS-FLI1* fusion transcript exhibited significant antitumor effects in a mouse model of Ewing's sarcoma (49). RNAi has also been used to target aberrant genes within cancer-related signaling pathways. For example, RNAi has been directed against activated *BRAF*, a mutant commonly associated with melanoma. In one study, a human melanoma cell line heterozygous for this mutation was transduced with either a lentiviral vector expressing a mutant-specific shRNA, an shRNA corresponding to the wild-type *BRAF* sequence, or a control shRNA. These cells were then used to generate xenografts in different cohorts of mice (132). Mice receiving either of the *BRAF* shRNAs exhibited smaller tumors than control mice, with the mutant-specific variant showing the least amount of tumor growth. In another study, an inducible *BRAF* shRNA was able to inhibit tumor growth in vivo and shrink pre-existing melanoma xenografts (43). RNAi has been used for the in vivo targeting of a number of other genes integral to signaling cascades. Some of these include *K-RAS* (study described above), *EGFR*, and *c-Met* (9, 119, 156).

RNAi has also been used to target genes that are differentially expressed in cancer. For example, Survivin is minimally expressed in normal cells, but frequently expressed in many tumors where it is thought to alter cell cycle regulation and suppress apoptosis. Several in vivo cancer model systems, including glioma, esophageal carcinoma, and rhabdomyosarcoma xenografts, have been used to assess the effect of *Survivin* downregulation (12, 139, 144). In all cases, a substantial inhibition of tumor growth was observed following RNAi against *Survivin*. In an analogous example, the induction of RNAi against telomerase, another gene preferentially expressed in tumor cells, showed substantial inhibition of tumor growth in xenografts of cervical and bladder cancers (96, 159). In one of these studies, RNAi enhanced the sensitivity of tumor cells to existing chemotherapeutic agents (96). The use of RNAi to identify these types of "synthetic lethal" combinations will likely be a very important field of study in coming years. Related to this is the potential for RNAi to inhibit proteins associated with chemotherapeutic resistance (for example, MDR1). The feasibility of such an approach was demonstrated by showing that an shRNA targeting *MDR1* was able to mitigate drug resistance in vivo (124). No matter the approach, it is likely that at least some RNAi-based clinical trials will be developed over the next few years, particularly for cancers where there are few existing treatment options.

CONCLUSIONS

The discovery of RNAi has dramatically changed our understanding of how gene expression is modulated. Furthermore, the ability to harness this mechanism through the use of artificial triggers has become an invaluable tool for the scientific community. RNAi has not only been applied to understand the functions of a limited number of genes in focused studies, but has been employed in larger, genome-wide applications. The information provided by these studies has significantly enhanced our ability to identify new disease-related molecular targets and rationally improve therapeutic development. Furthermore, RNAi-based technologies have, in themselves, the potential for clinical application. While there has been rapid progress in moving RNAi-based technologies into the clinic, a substantial number of issues still remain. Some of these issues are similar to those encountered by the gene therapy and antisense fields. Specifically, ensuring efficient delivery of nucleic acids with minimal stimulation of host responses is absolutely critical. Other issues relate to the possibility of overwhelming the endogenous RNAi machinery

and the potential downregulation of nontargeted transcripts. However, the enormous potential of RNAi makes overcoming these obstacles a worthwhile endeavor.

SUMMARY POINTS

1. RNAi is a conserved endogenous mechanism that can mediate gene silencing at a transcriptional and post-transcriptional level in mammalian cells. Artificial triggers of RNAi have been rapidly developed to modulate gene expression. Applications of RNAi have ranged from the single gene to genome-wide scales.
2. RNAi effectors can induce unintended effects, termed off-target effects. Off-target effects can result in the misinterpretation of RNAi-induced phenotypes. There are a number of steps that should be taken to minimize the impact of off-target effects.
3. RNAi has been used for large-scale functional screens in human cells. A number of elegant screening strategies have been described. These screens have yielded a wealth of information, ranging from a better understanding of biological processes to the identification of novel anticancer molecular targets.
4. A variety of RNAi-based strategies have been successfully developed to target genes in vivo. Although most studies have been conducted in preclinical models, there are currently a number of clinical trials involving RNAi-based therapeutics.

DISCLOSURE STATEMENT

NC collaborates with Qiagen Inc., Germantown, MD, on research investigating aspects of the use of synthetic siRNAs to mediate RNAi.

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