# nature reviews genetics

# Tools for studying and using small RNAs: from pathways to functions to therapies

Kenneth Chang and Gregory J. Hannon

During the past decade, small RNAs have emerged as crucial regulators of gene expression and genome function, having roles in almost every aspect of biology<sup>1</sup>. Many small RNAs act through RNA interference (RNAi)-related mechanisms, which involve programming the RNA-induced silencing complex (RISC) to recognize and repress targets. One class of small RNA, the microRNAs (miRNAs), naturally regulates programmes of gene expression. Altered miRNA function contributes to human disease, and manipulation of specific miRNAs is now being pursued as a novel therapeutic modality. Small RNAs have

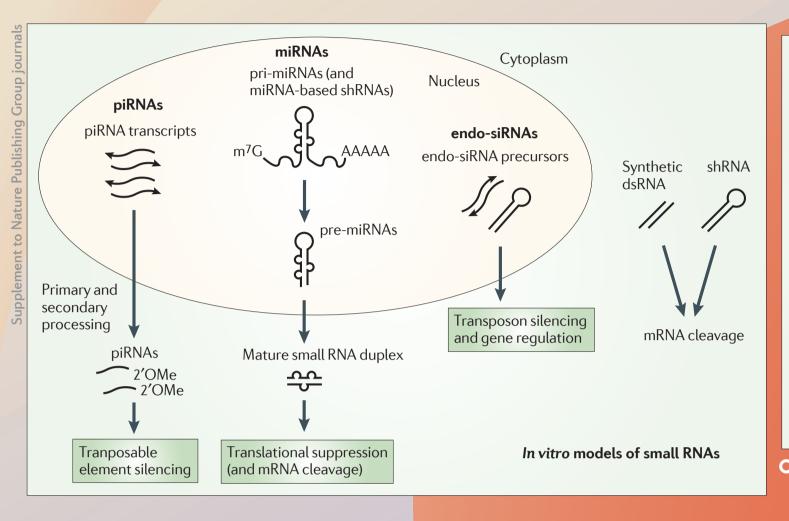
also been adapted for use as tools based on reprogramming the RNAi machinery to silence specific coding or non-coding RNAs. These tools have been exploited to investigate gene function in cultured cells and in living animals. Genome-scale collections of silencing triggers permit phenotype-based genetic screens to be carried out easily in organisms in which they were previously difficult or impossible. Such strategies are being used to discover and validate new therapeutic targets, and small RNAs themselves may offer a mechanism for inhibiting targets that are currently viewed as 'undruggable'.

IN VIVO RNAi SCREENS

Syngeneic recipient



**SMALL RNAs AS TOOLS** 



Microarray technology

#### **Small RNA biogenesis**

Currently, our understanding of small RNA biogenesis is most complete for small RNAs that exert their effects through RISC. In animals, three main classes of such small RNAs are recognized: small interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), and miRNAs<sup>1</sup>. siRNAs are derived from double-stranded precursors that are processed by the RNase III family enzyme. Dicer. In mammals, endogenous siRNAs are most abundant in germ cells, but in invertebrates they are more widespread. miRNA precursors contain short hairpin segments that contain the mature miRNA sequence. These precursors are processed through the serial action of two double-stranded RNAses (dsRNAses). Drosha and Dicer. piRNAs are mainly expressed in germ cells, in which they guard against the activity of transposons. Their biogenesis remains to be fully understood.

In vivo approaches Short hairpin RNAs (shRNAs) are particularly powerful tools for probing gene function in transgenic animals. Animals that are mosaic for knockdown of a gene of interest can be created by the transplantation of shRNA-modified stem cells<sup>23</sup>. Mice in which knockdown is achieved throughout the animal can also be produced<sup>24</sup>. This can be achieved using optimized shRNAs that are integrated into a specific genomic locus that is competent for expression in most tissues. This strategy offers the potential for

tissue-specific or regulated repression of nearly any gene in the tissue or cell type of interest.

Identify tumoriaenic shRNA by genomic (deep) sequencing

Small RNAs in therapy

Both single-gene and genome-wide approaches

classes that are amenable to conventional drug

discovery. Others will be among protein classes

small RNAs themselves offer a novel therapeutic

express shRNAs in specific disease contexts.

generally considered 'undruggable'. In these cases,

modality, although delivery of small RNAs to target

cells in vivo has proved challenging. Many groups are

also pursuing the use of gene therapy approaches to

discovery of new therapeutic targets for a variety of

diseases. Some of these targets will lie within protein

using small RNAs have great potential for the

Sensitized low tumorigenic background

Additional lesions

(such as Myc,  $Tp53^{-/-}$  or  $Pten^{-/-}$ )

#### Small RNA tools for screening Small RNA tools have revolutionized the

ability to scan genomes for proteins that have an effect on a specified phenotype. Libraries of long dsRNAs have been widely used in worms and Drosophila melanogaster cells, and collections of transgenic flies expressing dsRNA triggers are readily available<sup>17–19</sup>. In mammals, both genome-wide and subgenomic, focused libraries of synthetic siRNAs and shRNA expression constructs are widely used<sup>20-22</sup>. Screening can be carried out using each trigger individually, often using high-content measurement methods, or in multiplexed formats, which often use a

selective pressure to detect an impact on

The repressive potential of small RNA pathways can be

and enter RISC without the need for further processing<sup>11</sup>.

shRNAs must be expressed from a transiently delivered or

use but silence their targets only transiently, limited by the

permanent repression and the shRNAs themselves can be

repression levels and examination of reversible effects<sup>15,16</sup>.

expressed from regulated promoters, enabling tuning of

reoriented towards selected cellular genes, enabling studies of

gene function. In mammals, two types of RNAi triggers are widely

used. siRNAs are transiently delivered to cells, usually in culture,

genomically integrated vector and must be processed by Drosha

and Dicer or, in some cases, Dicer alone 12-14, siRNAs are simple to

persistence of the chemically synthesized trigger. shRNAs offer

more flexibility; integrated constructs can provide essentially

a specific process.

Candidate genes (for example, selected on the basis of cancer genome copy number information)

shRNAs targeting

candidate genes

Well-by-well screening Pooled (shRNA) screening

# In vitro

# In vitro RNAi screens

• shRNA libraries

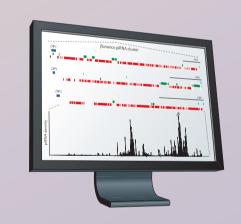
• dsRNA libraries

• siRNA libraries

#### **GENE-SILENCING TECHNOLOGIES**

#### **DISCOVERY**

Next-generation sequencing technologies



#### **Small RNA discovery** Small RNA discovery has been revolutionized by

Informatics resources

miRNAs and miRNA

target databases

next-generation sequencing. Following ligation of specific linkers to small RNAs, cDNAs can be produced, which are ideally suited to sequencing using short-read platforms. Such approaches have expanded the catalogue of small RNAs that are thought to act through RISC and have enabled the discovery of novel small RNA classes. Deep sequencing has also proved useful for monitoring the expression of annotated small RNAs, but alternative strategies, such as microarrays and quantitative PCR (qPCR), also provide economical alternatives. Databases now offer online catalogues of known small RNAs<sup>2</sup>.

**FUNCTION** 

Genetic systems

Knockouts

Transgenics

**Targeting small RNAs for therapeutics** Alterations in miRNAs themselves, or in their binding sites on crucial targets, have been associated with human disease. This presents an opportunity to agonize or antagonize specific miRNAs for therapeutic benefit. Several strategies have been developed to deliver antagomirs in vivo. These approaches have been validated in preclinical models, including rodents and primates, and some miRNA antagonists have entered human clinical trials<sup>25</sup>.

Antisense approaches

LNAs

Antagomirs

miRNA decov

Endogenous miRNA target

Seed complement

miRNA sponges

## Viral vectors

**DELIVERY** 

**THERAPY** 

**PRECLINICAL** 

**MODELS** 

Argonaute protein

Seed complement

Liposomes Nanoparticles

#### **Small RNA function**

Understanding the function of small RNAs often begins with studying their expression patterns. The identification of potential targets relies on the assumption that the small RNA and the target must share some sequence complementarity. Computational algorithms predict miRNA targets on the basis of the presumed character of miRNA-mRNA interactions and the conservation of their binding sites<sup>3,4</sup>. Several biochemical methods of target identification have also been developed, such as crosslinking immunoprecipitation (CLIP)<sup>5</sup> and tandem affinity purification of miRNA target mRNAs (TAP-Tar)<sup>6</sup>. Functional studies of small RNAs can rely on conventional genetic strategies, such as knockouts. Overexpression of miRNA and the use of miRNA antagonists are also popular approaches. Antagomirs are chemically synthesized miRNA-complementary oligonucleotides with modified chemical backbones that act as antisense inhibitors of miRNA function<sup>7–9</sup>. Genetically encoded 'decoys' called miRNA sponges that contain miRNA binding sites function similarly to antagomirs by titrating the miRNA away from its natural targets<sup>10</sup>. Model organisms, such as *Caenorhabditis* elegans, Drosophila melanogaster and zebrafish, have also proved to be key for understanding the function of conserved small RNAs.

## TOOLS FOR STUDYING SMALL RNAs

Horizon Discovery RNAi Solutions from Dharmacon™

Access the largest and most complete portfolio of innovative and technologically advanced RNAi tools for gene silencing. Since discovery of the endogenous RNAi pathway, our scientists have made key contributions to the field of RNAi that have been foundational to the development of potent, specific and highly functional siRNA, microRNA-adapted shRNA and microRNA reagents. Today, Horizon's RNAi products from Dharmacon facilitate a variety of applications including transient, long-term, inducible and in vivo RNAi strategies that extend from basic research in biology to improved therapeutic strategies in medicine.

- siRNA Patented dual-strand modifications in ON-TARGETplus™ siRNA provides unrivaled specificity and potency; Accell™ siRNA is the only siRNA reagent that can be delivered to difficult-to-transfect cells WITHOUT a transfection reagent
- SMARTvector™ shRNA microRNA-adapted shRNA for constitutive and inducible RNAi provide specific and potent, long-term silencing
- miRIDIAN™ microRNA Up-regulate or suppress endogenous mature microRNA function with rationally-designed synthetic and expressed microRNA mimic and hairpin inhibitors

In addition, our portfolio of molecular biology tools includes large collections of cDNAs and ORFs for gene overexpression and RNAi rescue, PCR and qPCR reagents, delivery reagents as well as validated protocols to support the entire RNAi workflow. Horizon Discovery continues to be the industry leader in the field of RNA chemistry, RNAi biology and high-throughput screening, and partners with the RNAi screening community through technical support and screening services. From single gene knockdown to genome-scale RNAi screens, find your RNAi solutions at https://horizondiscovery.com/en/navigation/gene-modulation/

#### **Abbreviations**

Types of small RNA tools

m<sup>7</sup>G, 7-methylguanosine cap; 2'OMe, 2'-O-methyl; MSCV, murine stem cell virus.

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