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Review

RNA interference as a gene knockdown technique

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ABSTRACT

Not many scientific breakthroughs bring significant advances simultaneously in both basic research and translational applications like the discovery of RNA interference. Along with the elucidation of the RNA interference pathway and the discovery of its participation in crucial biological events, a branch of science has grown to utilize the RNA interference pathway as a biotechnology for both basic and applied research. Small interference RNA, plasmid-, and virus-encoded short-hairpin RNA are now regular reagents in the tool box of biologists to knockdown the expression of specific genes posttranscriptionally. Efforts have also been made to develop RNA interference based therapeutics into reality. Many concerns about the RNA interference technique have now been answered through research and development, although hurdles are still present. In this review, the RNA interference/microRNA pathway is briefly introduced followed with a detailed summary about the design and application of the RNA interference experiments, along with examples of the utilization of the RNA interference technology in animal cells and model organisms. Recent progresses and current concerns are also highlighted. Two techniques, namely morpholino and external guide sequence, are discussed as complementary gene knockdown technology. RNA interference technology, along with several other alternative gene knockdown techniques, is now indispensable to modern biological and medical research.

Contents

1. Introduction

Fire, Mello and co-workers published their seminal work of RNA interference (RNAi) about a decade ago by revealing double-stranded RNA (dsRNA) as the trigger of post-transcriptional silencing in *Caenorhabditis elegans* (Fire et al., 1998). Another phenomenon of post-transcriptional silencing comes from microRNA (miRNA). In 1993, Ambros, Ruvkun and co-workers cloned the first short non-coding RNA (later called microRNA collectively), *lin-4*, also in *C. elegans*; and showed that *lin-4* potentially functions by binding to the 3′ UTR of its target, *lin-14*, through partial complementary sequences (Lee et al., 1993; Wightman et al., 1993). Advancement in the research eventually merged the RNAi pathway with the miRNA pathway by showing that core components are closely shared (Fig. 1; Murchison and Hannon, 2004).

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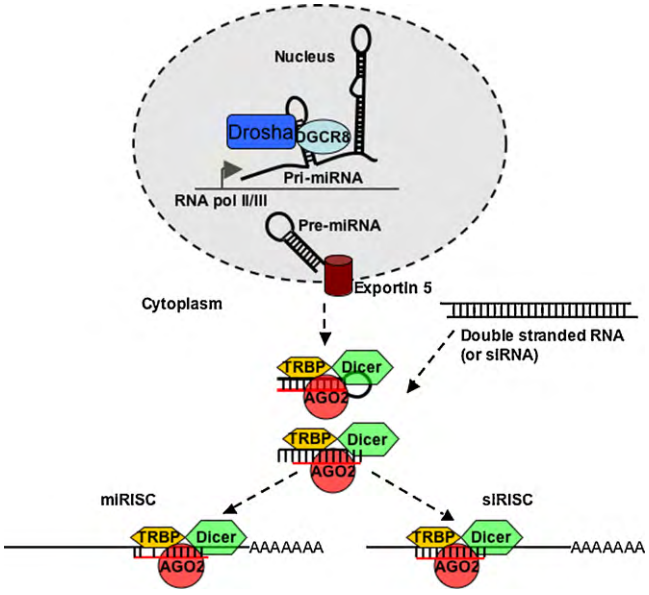

Fig. 1. The RNAi/miRNA pathway. miRNA genes are predominately transcribed by RNA polymerase II (some are transcribed by RNA polymerase III) into large primary miRNA (pri-miRNA) transcripts with poly(A) tail and 5′ cap. Pri-miRNAs are processed by a complex (called microprocessor) of DGCR8 and Drosha into miRNA precursors (pre-miRNAs) of ~70 nt with a hairpin structure of stem-loop. The formation of some pre-miRNAs bypasses the Drosh/DGCR8 because they are processed as intron (mirtron) in the pre-mRNA splicing (Ruby et al., 2007; Okamura et al., 2007). Pre-miRNAs are exported from nucleus into cytoplasm by Exportin 5, and get loaded into a complex composite of Dicer, Ago2, TRBP, and other known or unknown proteins. Double-stranded RNA (dsRNA) can also be loaded into this complex for processing. Pre-miRNAs or dsRNAs are cut into short miRNA duplex or small interference RNA (siRNA) around 21 bp by Dicer, and one strand (the guide strand, in red) is then be integrated into RNA-induced silencing complex (RISC) whose core component is Ago2. Another strand (the passenger strand, in black) will be degraded (for the siRNA duplex) or released (for the miRNA duplex). siRNA can also be loaded directly into the RISC complex without the need to slice by Dicer. Inside the siRISC (small interference RNA programmed RNA-induced silencing complex), the siRNA guide strand, by complete complementation with the targeted mRNA, triggers the degradation of mRNA in the RISC; whereas inside the miRISC (miRNA programmed RNA-induced silencing complex), miRNA generally suppresses the translation of the target mRNA by incomplete complementation with the 3′ UTR region. The whole pre-miRNA processing complex and RISC may be dynamic, share common components, and demand further characterization.

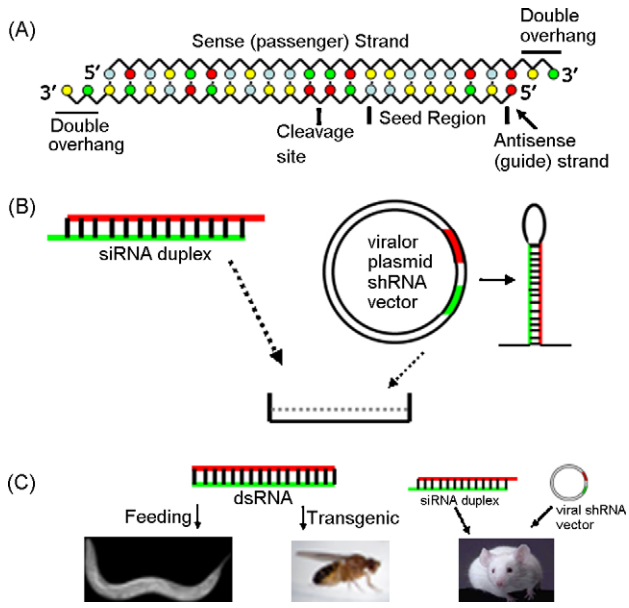

Fig. 2. Strategies of the RNAi technology. (A) Typical composition of a siRNA duplex with the passenger strand, the guide strand, seed region, and the cleavage site labeled. The first nucleotide (5′) of the guide strand is generally unpaired. siRNA duplexes can be chemically modified or conjugated for the purposes of increasing stability, avoiding immunostimulation, improving delivery, and enhancing potency. (B) siRNA duplexes or genetically encoded shRNA can be transfected (or transformed for viral shRNA) into mammalian cells with different methods. *Drosophila* S2 cells can also be transfected with dsRNA for RNAi knockdown. High throughput, whole-genome scale screening can be performed with cell cultures using siRNA, plasmid shRNA, or viral shRNA libraries. (C) RNAi can be applied to whole animals in *C. elegans*, *Drosophila*, and mice. Bacteria expressing dsRNA coded by a plasmid can be fed to *C. elegans* for RNAi knockdown; whereas dsRNA encoded by transgene can be induced to express in *Drosophila*. siRNA and viral shRNA can by applied to mice, and proper chemical modifications and delivery strategies should be considered. High throughput, whole-genome scale screening can also be done at whole animal level for *C. elegans* and *Drosophila*.

In principle, dsRNA, or microRNA precursor is cut into short interference RNA (siRNA) or miRNA duplex around 21 nucleotides (nt) by Dicer and its associated proteins such as TRBP. One strand (the guide strand, Figs. 1 and 2) is then be integrated into RISC. RISC is RNA-induced silencing complex whose core component is Ago2. Another strand (the passenger strand) will be degraded (for the siRNA duplex) or released (for the miRNA duplex). The siRNA guide strand, by complete complementation with the targeted mRNA, triggers the degradation of mRNA in the RISC; whereas miRNA generally suppresses the translation of the target mRNA by incomplete complementation with the 3′ UTR region (Fig. 1; Preall and Sontheimer, 2005).

Upon the discovery that dsRNA can be introduced exogenously into eukaryotic cells to knockdown target mRNAs in a sequence specific manner, a lot of laboratories started to design strategies to synthesis long dsRNA for later transfection experiments, or to construct plasmids encoding dsRNA for the knocking down of genes with special interest (Clemens et al., 2000; Tavernarakis et al., 2000; Montgomery et al., 1998). These attempts were largely successful in *C. elegans* and *Drosophila*, while inefficient in mammalian cells (Ui-Tei et al., 2000; Caplen et al., 2000). Reports also came out about long dsRNA triggering innate immune responses and cytotoxicities (Stark et al., 1998; Minks et al., 1979). Upon the discovery that RNA interference is mediated by ~21 nt small RNAs, and further the demonstration that siRNA duplex ~21 base pairs (bp) long can be applied to knock down gene expression in both *Drosophila* and mammalian cell cultures without triggering unwanted immune responses and cytotoxicity by Tuschl and co-workers, the RNAi technique became more practical (Zamore et al., 2000; Elbashir et al., 2001a,b). Today, the majority of RNAi knockdown experiments are performed directly with siRNAs or plasmid-/virus-encoded RNAs that eventually give rise to siRNA *in vivo*.

2. Design of siRNA

There are currently two ways to harness the endogenous RNAi pathway for gene knockdown purposes: either by introducing a viral or plasmid vector to express short hairpin (shRNA) that would then be processed by Dicer into siRNA (Abbas-Terki et al., 2002; Kunath et al., 2003); or by delivering directly siRNA into the cytoplasm (Elbashir et al., 2001a,b; Fig. 2). shRNA, which mimics a miRNA precursor, is usually expressed under a mammalian H1 or U6 promoter in a viral or plasmid vector. For either method, RNAi technique starts from the choosing and designing of one or several functional siRNAs. The first consideration is to ensure the siRNA targets specifically to the mRNA of interest without unwanted off-target effects (specificity), and at the same time, the siRNA should have a desirable (if not the highest possible) knockdown efficiency (potency).

The initial siRNA design starts with a bioinformatics-aided search for 'targetable' sequences of ~21 nt long in the mRNA of interest (Pei and Tuschl, 2006). Because a perfect complement with the targeted mRNA triggers degradation, and an imperfect comple-