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(54) **RNA INTERFERENCE MEDIATING SMALL RNA MOLECULES**

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(58) **Field of Classification Search** 436/6; 435/91.1, 91.3, 325, 6; 514/44; 536/23.1, 536/24.5

See application file for complete search history.

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(57) **ABSTRACT**

Double-stranded RNA (dsRNA) induces sequence-specific post-transcriptional gene silencing in many organisms by a process known as RNA interference (RNAi). Using a *Drosophila* in vitro system, we demonstrate that 19–23 nt short RNA fragments are the sequence-specific mediators of RNAi. The short interfering RNAs (siRNAs) are generated by an RNase III-like processing reaction from long dsRNA. Chemically synthesized siRNA duplexes with overhanging 3' ends mediate efficient target RNA cleavage in the lysate, and the cleavage site is located near the center of the region spanned by the guiding siRNA. Furthermore, we provide evidence that the direction of dsRNA processing determines whether sense or antisense target RNA can be cleaved by the produced siRNP complex.

15 Claims, 26 Drawing Sheets

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The invention claimed is:

1. A method for preparing a double stranded RNA molecule which mediates the cleavage of an mRNA in a mammalian cell, comprising

(a) synthesizing two RNA strands each having a length from 19–25 nucleotides, and

(b) combining the synthesized RNA strands under conditions suitable to form a double stranded RNA molecule, wherein said double stranded RNA molecule has a double stranded region of 14–24 nucleotides in length and one or two 3' overhang regions of 1–5 nucleotides in length.

2. The method according to claim 1, wherein said RNA strands have a length of 21–24 nucleotides in length.

3. The method according to claim 2, wherein said overhang regions are 2–4 nucleotides in length.

4. The method according to claim 1, wherein the RNA strands are chemically synthesized.

5. The method according to claim 1, wherein the RNA strands are enzymatically synthesized.

6. A method for preparing a double stranded RNA molecule which mediates the cleavage of a target mRNA in a mammalian cell, comprising

(a) selecting a target mammalian mRNA sequence or target gene sequence,

(b) synthesizing a first RNA strand having a length from 19–25 nucleotides, wherein said first RNA strand is complementary to contiguous nucleotides in said target mammalian mRNA or said target gene sequence

(c) synthesizing a second RNA strand having a length from 19–25 nucleotides, wherein said second RNA strand is complementary to 14–24 nucleotides from said first RNA strand, and

(d) combining the first and second RNA strands under conditions suitable to form a double stranded RNA molecule, wherein said double stranded RNA molecule has a double stranded region of 14–24 nucleotides in length and one or two 3' overhang regions of 1–5 nucleotides in length.

7. An improved method for preparing a double stranded RNA molecule for mediating the cleavage of an mRNA in a mammalian cell, comprising synthesizing a double stranded RNA molecule,

wherein the improvement comprises synthesizing a double stranded RNA molecule having a double stranded region of 16–24 nucleotides in length and one or more 3' overhang regions of 1–3 nucleotides in length.

8. An improved method for preparing a double stranded RNA molecule for mediating the cleavage of an mRNA in a mammalian cell, comprising preparing and isolating a double stranded RNA molecule,

wherein the improvement comprises preparing and isolating a double stranded RNA molecule having a double stranded region of 16–24 nucleotides in length and one or more 3' overhang regions of 1–3 nucleotides in length.

9. A method for preparing a double stranded RNA molecule which mediates the cleavage of an mRNA in a mammalian cell, comprising

(a) synthesizing two RNA strands each having a length from 19–23 nucleotides, and

(b) combining the synthesized RNA strands under conditions suitable to form a double stranded RNA molecule, wherein said double stranded RNA molecule has a double stranded region of 14–22 nucleotides in length and one or two 3' overhang regions of 1–5 nucleotides in length.

10. The method according to claim 9, wherein said overhang regions are 2–4 nucleotides in length.

11. The method according to claim 9, wherein the RNA strands are chemically synthesized.

12. The method according to claim 9, wherein the RNA strands are enzymatically synthesized.

13. A method for preparing a double stranded RNA molecule which mediates the cleavage of a target mRNA in a mammalian cell, comprising

(a) selecting a target mammalian mRNA sequence or target gene sequence,

(b) synthesizing a first RNA strand having a length from 19–23 nucleotides, wherein said first RNA strand is complementary to contiguous nucleotides in said target mammalian mRNA or said target gene sequence

(c) synthesizing a second RNA strand having a length from 19–23 nucleotides, wherein said second RNA strand is complementary to 16–22 nucleotides from said first RNA strand, and

(d) combining the first and second RNA strands under conditions suitable to form a double stranded RNA molecule, wherein said double stranded RNA molecule has a double stranded region of 16–22 nucleotides in length and one or two 3' overhang regions of 1–5 nucleotides in length.

14. An improved method for preparing a double stranded RNA molecule for mediating the cleavage of an mRNA in a mammalian cell, comprising synthesizing a double stranded RNA molecule,

wherein the improvement comprises synthesizing a double stranded RNA molecule having a double stranded region of 16–22 nucleotides in length and one or more 3' overhang regions of 1–3 nucleotides in length.

15. An improved method for preparing a double stranded RNA molecule for mediating the cleavage of an mRNA in a mammalian cell, comprising preparing and isolating a double stranded RNA molecule,

wherein the improvement comprises preparing and isolating a double stranded RNA molecule having a double stranded region of 16–22 nucleotides in length and one or more 3' overhang regions of 1–3 nucleotides in length.

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