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***Tuschl*, et al.** **July 18, 2006**

RNA interference mediating small RNA molecules

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.

Inventors: ***Tuschl*; Thomas** (New York, NY)**; Elbashir; Sayda Mahgoub** (Cambridge, MA)**; Lendeckel; Winfried** (Hohengendern, **DE**)

Assignee: **Max-Planck-Gesellschaft zur Foerderung der Wissenschaften, e.V.** (Munich, **DE**)

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Primary Examiner: Wang; Andrew
Assistant Examiner: Bowman; Amy H.
Attorney, Agent or Firm: Rothwell, Figg, Ernst & Manbeck, P.C.

Claims

The invention claimed is:

1. A method of preparing a double-stranded RNA molecule, wherein each RNA strand has a length from 19 25 nucleotides, wherein said RNA molecule is capable of target-specific nucleic acid modifications and wherein at least one strand has a 3'-overhang of 1 5 nucleotides, comprising (a) synthesizing two RNA strands each having a length from 19 25 nucleotides, wherein said RNA strands are capable of forming a double-stranded RNA molecule, (b) combining the synthesized RNA strands under conditions, wherein a double-stranded RNA molecule which mediates target-specific nucleic acid modifications is formed, wherein said double-stranded RNA molecule consists of a single double stranded region and single stranded regions of 1 to 5 nucleotides at the 3' ends of at least one of the strands of said double-stranded RNA molecule.

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

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

4. The method of claim 1, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 5 nucleotides.

5. The method of claim 1, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

6. The method of claim 1, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

7. The method of claim 1, wherein each strand has a length from 20 22 nucleotides.

8. The method of claim 7, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 5 nucleotides.

9. The method of claim 7, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

10. The method of claim 7, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

11. The method of claim 1, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, N(R).sub.2 or CN, wherein R is C.sub.1 C.sub.6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I.

12. The method of claim 1, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phosrhorothioate group.

13. A method of preparing a double-stranded RNA molecule, wherein each strand has a length of from 19 25 nucleotides, wherein said RNA molecule is capable of mediating the cleavage of a target mRNA in a mammal and at least one strand has a 3' overhang of 1 3 nucleotides, comprising the steps of: a) selecting a target mammalian mRNA 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 16 24 nucleotides from said first RNA strand, and (d) combining the synthesized RNA strands under conditions suitable to form a double stranded RNA molecule, wherein said double stranded RNA molecule consists of a single double stranded region of from 16 24 nucleotides in length and one or two single stranded 3' overhang regions of 1 3 nucleotides in length each.

14. The method of claim 13, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

15. The method of claim 13, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

16. The method of claim 13, wherein each strand has a length from 20 22 nucleotides.

17. The method of claim 16, wherein both strands of said double-stranded RNA each

have a 3'-overhang from 1 3 nucleotides.

18. The method of claim 16, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

19. The method of claim 13, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, N(R).sub.2 or CN, wherein R is C.sub.1 C.sub.6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I.

20. The method of claim 13, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phoshorothioate group.

21. A method of preparing a double-stranded RNA molecule, wherein each RNA strand has a length from 19 23 nucleotides, wherein said RNA molecule is capable of target-specific nucleic acid modifications and wherein at least one strand has a 3'-overhang of 1 5 nucleotides, comprising (c) synthesizing two RNA strands each having a length from 19 23 nucleotides, wherein said RNA strands are capable of forming a double-stranded RNA molecule, (d) combining the synthesized RNA strands under conditions, wherein a double-stranded RNA molecule which mediates target-specific nucleic acid modifications is formed, wherein said double-stranded RNA molecule consists of a single double stranded region and single stranded regions of 1 to 5 nucleotides at the 3' ends of at least one of the strands of said double-stranded RNA molecule.

22. The method according to claim 21, wherein the RNA strands are chemically synthesized.

23. The method according to claim 21, wherein the RNA strands are enzymatically synthesized.

24. The method of claim 21, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 5 nucleotides.

25. The method of claim 21, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

26. The method of claim 21, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

27. The method of claim 21, wherein each strand has a length from 20 22 nucleotides.

28. The method of claim 27, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 5 nucleotides.

29. The method of claim 27, wherein both strands of said double-stranded RNA each

have a 3'-overhang from 1 3 nucleotides.

30. The method of claim 21, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

31. The method of claim 21, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, N(R).sub.2 or CN, wherein R is C.sub.1 C.sub.6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I.

32. The method of claim 21, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phosphorothioate group.

33. A method of preparing a double-stranded RNA molecule, wherein each strand has a length of from 19 23 nucleotides, wherein said RNA molecule is capable of mediating the cleavage of a target mRNA in a mammal and at least one strand has a 3 overhang of 1 3 nucleotides, comprising the steps of: a) selecting a target mammalian mRNA 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 synthesized RNA strands under conditions suitable to form a double stranded RNA molecule, wherein said double stranded RNA molecule consists of a single double stranded region of from 16 22 nucleotides in length and one or two single stranded 3' overhang regions of 1 3 nucleotides in length each.

34. The method of claim 33, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

35. The method of claim 33, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

36. The method of claim 33, wherein each strand has a length from 20 22 nucleotides.

37. The method of claim 36, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

38. The method of claim 36, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

39. The method of claim 33, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, N(R).sub.2 or CN, wherein R is C.sub.1 C.sub.6 alkyl, alkenyl or alkynyl and halo

is F, Cl, Br or I.

40. The method of claim 33, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phosphorothioate group.

41. A method of preparing a double-stranded RNA molecule, wherein each RNA strand has a length from 19 25 nucleotides, wherein said RNA molecule is capable of target-specific nucleic acid modifications and wherein at least one strand has a 3'-overhang of 1 5 nucleotides, comprising (a) synthesizing two RNA strands each having a length from 19 25 nucleotides, wherein said RNA strands are capable of forming a double-stranded RNA molecule, (b) combining the synthesized RNA strands under conditions, wherein a double-stranded RNA molecule which mediates target-specific nucleic acid modifications is formed, wherein the only single stranded regions in said RNA molecule are single stranded regions of 1 to 5 nucleotides at the 3' ends of at least one of the strands of said double-stranded RNA molecule.

42. The method according to claim 41, wherein the RNA strands are chemically synthesized.

43. The method according to claim 41, wherein the RNA strands are enzymatically synthesized.

44. The method of claim 41, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 5 nucleotides.

45. The method of claim 41, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

46. The method of claim 41, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

47. The method of claim 41, wherein each strand has a length from 20 22 nucleotides.

48. The method of claim 47, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 5 nucleotides.

49. The method of claim 47, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

50. The method of claim 47, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

51. The method of claim 41, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, N(R).sub.2 or CN, wherein R is C.sub.1 C.sub.6 alkyl, alkenyl or alkynyl and halo

is F, Cl, Br or I.

52. The method of claim 41, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phosphorothioate group.

53. A method of preparing a double-stranded RNA molecule, wherein each strand has a length of from 19 25 nucleotides, wherein said RNA molecule is capable of mediating the cleavage of a target mRNA in a mammal and at least one strand has a 3' overhang of 1 3 nucleotides, comprising the steps of: a) selecting a target mammalian mRNA 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 16 24 nucleotides from said first RNA strand, and (d) combining the synthesized RNA strands under conditions suitable to form a double stranded RNA molecule, wherein said double stranded RNA molecule is 16 24 nucleotides in length and the only single stranded regions in said RNA molecule are one or two single stranded 3' overhang regions of 1 3 nucleotides in length each.

54. The method of claim 53, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

55. The method of claim 53, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

56. The method of claim 53, wherein each strand has a length from 20 22 nucleotides.

57. The method of claim 56, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

58. The method of claim 56, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

59. The method of claim 53, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, N(R).sub.2 or CN, wherein R is C.sub.1 C.sub.6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I.

60. The method of claim 53, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phosphorothioate group.

61. A method of preparing a double-stranded RNA molecule, wherein each RNA strand has a length from 19 23 nucleotides, wherein said RNA molecule is capable of target-specific nucleic acid modifications and wherein at least one strand has a 3'-overhang of 1 5 nucleotides, comprising a. synthesizing two RNA strands each having a length from 19

23 nucleotides, wherein said RNA strands are capable of forming a double-stranded RNA molecule, b. combining the synthesized RNA strands under conditions, wherein a double-stranded RNA molecule which mediates target-specific nucleic acid modifications is formed, wherein the only single stranded regions in said RNA molecule are single stranded regions of 1 to 5 nucleotides at the 3' ends of at least one of the strands of said double-stranded RNA molecule.

62. The method according to claim 61, wherein the RNA strands are chemically synthesized.

63. The method according to claim 61, wherein the RNA strands are enzymatically synthesized.

64. The method of claim 61, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 5 nucleotides.

65. The method of claim 61, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

66. The method of claim 61, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

67. The method of claim 61, wherein each strand has a length from 20 22 nucleotides.

68. The method of claim 67, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 5 nucleotides.

69. The method of claim 67, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

70. The method of claim 67, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

71. The method of claim 61, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, N(R).sub.2or CN, wherein R is C.sub.1 C.sub.6alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I.

72. The method of claim 61, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phosphorothioate group.

73. A method of preparing a double-stranded RNA molecule, wherein each strand has a length of from 19 23 nucleotides, wherein said RNA molecule is capable of mediating the cleavage of a target mRNA in a mammal and at least one strand has a 3' overhang of 1 3 nucleotides, comprising the steps of: (a) selecting a target mammalian mRNA 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 synthesized RNA strands under conditions suitable to form a double stranded RNA molecule, wherein said double stranded RNA molecule is 6 22 nucleotides in length and the only single stranded regions in said RNA molecule are one or two single stranded 3' overhang regions of 1 3 nucleotides in length each.

74. The method of claim 73, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

75. The method of claim 73, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

76. The method of claim 73, wherein each strand has a length from 20 22 nucleotides.

77. The method of claim 76, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1 3 nucleotides.

78. The method of claim 76, wherein both strands of said double-stranded RNA eachhave a 3'-overhang of 2 nucleotides.

79. The method of claim 73, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH.sub.2, NHR, N(R).sub.2 or CN, wherein R is C.sub.1 C.sub.6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I.

80. The method of claim 73, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phosphorothioate group.