CLAIMS

1. A stealth RNA gene expression system comprising:

a negative-sense single-stranded RNA (A),

a single-stranded RNA binding protein (B), and

an RNA-dependent RNA polymerase (C),

wherein the stealth RNA gene expression system is a complex that does not activate an innate immune system,

wherein the negative-sense single-stranded RNA (A) comprises RNA sequences (1) to (8):

(1) RNA sequences encoding any given protein or functional RNA,

(2) RNA sequences constituting noncoding region(s), the RNA sequences (2) having a length of 5 nucleotides to 49 nucleotides and being fragments of mRNA(s) expressed in animal cells,

(3) transcription start signal sequences recognized by the RNA-dependent RNA polymerase,

(4) transcription termination signal sequences recognized by the RNA-dependent RNA polymerase,

(5) RNA sequences containing replication origins recognized by the RNA-dependent RNA polymerase,

(6) RNA sequences encoding the RNA-dependent RNA polymerase with codons optimized for a biological species of cells for transfection,

(7) an RNA sequence encoding a protein that regulates

activity of the RNA-dependent RNA polymerase with codons optimized for the biological species of the cells for transfection, and

(8) an RNA sequence encoding the single-stranded RNA binding protein with codons optimized for the biological species of cells for transfection,

wherein the RNA-dependent RNA polymerase encoded by the RNA sequences of the (6) consists of L protein and P protein of an RNA virus belonging to a paramyxovirus family,

wherein the protein that regulates activity of the RNA-dependent RNA polymerase encoded by the RNA sequence of the (7) is C protein of the RNA virus belonging to the paramyxovirus family,

wherein the single-stranded RNA binding protein encoded by the RNA sequence of the (8) is NP protein of the RNA virus belonging to the paramyxovirus family,

wherein all of the RNA sequences of the (3) to (5) are RNA sequences comprising a transcription start signal, a transcription termination signal, or a replication origin sequence isolated from a genome of the same virus as the RNA virus, and

wherein the RNA sequences encoding the L protein, P protein, C protein and NP protein are optimized for human cells, and have a GC content adjusted within a range of 50

to 60%.

2. The stealth RNA gene expression system according to claim 1, wherein the RNA sequences of the (1) comprise at least six genes, or are RNA sequences having a total length of 5000 or more nucleotides.

3. The stealth RNA gene expression system according to claim 1, wherein the RNA sequences of the (2) are fragments of mRNA(s) expressed in human cells.

4. The stealth RNA gene expression system according to claim 1, wherein each of the RNA sequences of the (2) has sequences identical to or different from one another and is adjacent to the 3′ terminal site and/or the 5′ terminal site of each of gene sequences comprised in the RNA sequences of the (1).

5. The stealth RNA gene expression system according to claim 1, wherein the RNA virus belonging to a paramyxovirus family is an RNA virus selected from the group consisting of Sendai virus, human parainfluenza virus, and Newcastle disease virus.

6. The stealth RNA gene expression system according to

claim 1,

wherein the transcription start signal sequences of the (3) are RNA sequences selected from the group of RNA sequences consisting of 3′-UCCCACUUUC-5′ (SEQ ID NO: 1), 3′-UCCCUAUUUC-5′ (SEQ ID NO: 2), 3′-UCCCACUUAC-5′ (SEQ ID NO: 3), 3′-UCCUAAUUUC-5′ (SEQ ID NO: 7), and 3′-UGCCCAUCUUC-5′ (SEQ ID NO: 9), and

wherein the transcription termination signal sequences of the (4) are RNA sequences selected from the group of RNA sequences consisting of 3′-AAUUCUUUUU-5′ (SEQ ID NO: 4), 3′-CAUUCUUUUU-5′ (SEQ ID NO: 5), 3′-UAUUCUUUUU-5′ (SEQ ID NO: 6), and 3′-UUAUUCUUUUU-5′ (SEQ ID NO: 8).

7. The stealth RNA gene expression system according to claim 4,

wherein each of the transcription start signal sequences of the (3) having sequence identical to or different from one another is adjacent to the 3′ terminal site of each of the RNA sequences of the (2) that is adjacent to the 3′ terminal site of each of gene sequences comprised in the RNA sequences of the (1), and

wherein each of the transcription termination signal sequences of the (4) is adjacent to the 5′ terminal site of the RNA sequence that is adjacent to the 5′ terminal site of each of gene sequences comprised in the RNA sequences of

the (1).

8. The stealth RNA gene expression system according to claim 5, wherein the RNA sequences comprising replication origins of the (5) comprise the following sequences (a), (b), (c) and (d):

(a) either the RNA sequence of 3′-UGGUCUGUUCUC-5′ (SEQ ID NO: 11) or 3′-UGGUUUGUUCUC-5′ (SEQ ID NO: 12),

(b) either the RNA sequence represented by 3′-GAGAACAGACCA-5′ (SEQ ID NO: 13) or 3′-GAGAACAAACCA-5′ (SEQ ID NO: 14),

(c) the RNA sequence represented by 3′-$(CNNNNN)_3$-5′ (SEQ ID NO: 15), and

(d) the RNA sequence represented by 3′-$(NNNNNG)_3$-5′ (SEQ ID NO: 16).

9. The stealth RNA gene expression system according to claim 8, wherein the RNA sequence of the (a) is positioned at the 3′ terminus of the negative-sense single-stranded RNA (A), and the RNA sequence of the (b) is positioned at the 5′ terminus.

10. The stealth RNA gene expression system according to claim 8, wherein the RNA sequence of the (c) starts at 79th nucleotide from the 3′ terminus of the negative-sense single-stranded RNA (A), and the RNA sequence of the (d)

starts at 96th nucleotide from 5′ terminus.

11. The stealth RNA gene expression system according to claim 8, wherein the RNA sequences comprising replication origins of the (5) further comprise, in a position of 97th to 116th nucleotides from the 3′ terminus of the negative-sense single-stranded RNA (A), the RNA sequence of (e) 3′-AAAGAAACGACGGUUUCA-5′ (SEQ ID NO: 17) or an RNA sequence having the same length of 18 nucleotides as the (e).

12. A stealth RNA vector comprising:

a complex comprising the stealth RNA gene expression system according to claim 1,

wherein the stealth RNA vector is capable of introducing the complex into animal cells, and

wherein the stealth RNA vector does not activate an innate immune system.

13. The stealth RNA vector according to claim 12, wherein the stealth RNA vector forms a virus particle capable of infecting animal cells.

14. An isolated animal cell transfected with the stealth RNA vector according to claim 12.

15. A stealth RNA which is a negative-sense single-stranded RNA (A) comprising:

(1) RNA sequences encoding any given protein or functional RNA,

(2) RNA sequences constituting noncoding region(s) that are unrecognizable by an innate immune system,

(3) transcription start signal sequences recognized by an RNA-dependent RNA polymerase,

(4) transcription termination signal sequences recognized by the RNA-dependent RNA polymerase,

(5) RNA sequences comprising replication origins recognized by the RNA-dependent RNA polymerase,

(6) RNA sequences encoding the RNA-dependent RNA polymerase and having a structure optimized to be unrecognizable by an innate immune system,

(7) an RNA sequence encoding a protein that regulates activity of the RNA-dependent RNA polymerase, and having a structure optimized to be unrecognizable by an innate immune system, and

(8) an RNA sequence encoding a single-stranded RNA binding protein and having a structure optimized to be unrecognizable by an innate immune system;

wherein the stealth RNA is capable of forming a complex that does not activate an innate immune system together with the single-stranded RNA binding protein (B), and the RNA-

dependent RNA polymerase (C),

wherein the RNA sequences of the (2) are RNA sequences having a length of 5 nucleotides to 49 nucleotides, and are fragments of mRNA(s) expressed in animal cells,

wherein the RNA-dependent RNA polymerase encoded by the RNA sequences of the (6) consists of L protein and P protein of an RNA virus belonging to a paramyxovirus family,

wherein the protein that regulates activity of the RNA-dependent RNA polymerase encoded by the RNA sequence of the (7) is C protein of the RNA virus belonging to the paramyxovirus family,

wherein the single-stranded RNA binding protein encoded by the RNA sequence of the (8) is NP protein of the RNA virus belonging to the paramyxovirus family from the same virus as the RNA virus,

wherein all of the RNA sequences of the (3) to (5) are RNA sequences comprising a transcription start signal, a transcription termination signal, or a replication origin sequence isolated from a genome of the same virus as the RNA virus, and

wherein the RNA sequences encoding the L protein, P protein, C protein and NP protein are optimized for human cells, and have a GC content adjusted within a range of 50 to 60%.

16. The stealth RNA according to claim 15,

wherein RNA sequences containing replication origins recognized by the RNA-dependent RNA polymerase of the (5) are located at the 3′ terminal site and the 5′ terminal site of the negative-sense single-stranded RNA (A), and

wherein the RNA sequence located at the 3' terminal site and the RNA sequence located at the 5' terminal site comprise RNA sequences complementary to each other.

17. The stealth RNA according to claim 15,

wherein each of the transcription start signal sequences of the (3) having sequences identical to or different from one another is placed adjacent to the 3′ terminal site of each of the RNA sequences of the (2) that is adjacent to the 3′ terminal site of each of plural gene sequences comprised in the RNA sequences of the (1), and

wherein each of the transcription termination signal sequences of the (4) is adjacent to the 5′ terminal site of the RNA sequence that is adjacent to the 5′ terminal site of each of plural gene sequences comprised in the RNA sequence of the (1).

18. The stealth RNA according to claim 15,

wherein each of the transcription start signal sequences of the (3) having sequences identical to or

different from one another is adjacent to the 3′ terminal site of each of the RNA sequences of the (2) that is adjacent to the 3′ terminal site of each of plural gene sequences comprised in the RNA sequences of the (1),

wherein each of the transcription termination signal sequences of the (4) is adjacent to the 5′ terminal site of the RNA sequence that is adjacent to the 5′ terminal site of each of plural gene sequences comprised in the RNA sequences of the (1),

wherein both of the transcription start signal sequences of the (3) and the transcription start signal sequences of the (4) constitute a cassette structure, together with restriction sites located at both ends of the cassette that can be cleaved by plural restriction endonucleases, and

wherein plural cassette structures are bound to each other.

19. A method for reconstituting a stealth RNA gene expression system, comprising:

(1) preparing an Escherichia coli expressing T7 RNA polymerase;

(2) introducing into the Escherichia coli host of the (1), at least a vector for Escherichia coli carrying an RNA encoding an RNA-dependent RNA polymerase and an RNA binding

protein, and a vector for Escherichia coli for expressing a DNA encoding RNA binding protein, together with the negative-sense single-stranded RNA (A) according to claim 1 to transform the host,

(3) forming a complex of the negative-sense single-stranded RNA comprising exogenous gene RNA expressed by T7 RNA polymerase, and RNA binding protein in the transformed Escherichia coli of the (2),

(4) preparing animal cells in which an RNA-dependent RNA polymerase is expressed, and

(5) introducing the complex of the negative-sense single-stranded RNA and the RNA binding protein obtained in the (3) into an animal cell host of the (4) to reconstitute a stealth RNA gene expression system comprising the negative-sense single-stranded RNA, and the complex of the RNA binding protein and the RNA-dependent RNA polymerase.