

Claims

[Claim 1]

A fusion protein for improving a protein expression level from a target mRNA, the fusion protein comprising:

(A) one or more functional domains which improve a protein expression level from an mRNA; and

(B) a polypeptide moiety which can bind to a target mRNA in an RNA base-selective or RNA base sequence-specific manner,

wherein polypeptide moiety (B) is a polypeptide moiety comprising one or more pentatricopeptide repeat (PPR) motifs, each PPR motif comprising a polypeptide consisting of 30 to 38 amino acids in length and being represented by Formula 1:

[Formula 1]

(Helix A)-X-(Helix B)-L (Formula 1)

where

Helix A is a moiety which consists of 12 amino acids in length and can form an α -helix structure, and is represented by Formula 2:

[Formula 2]

A₁-A₂-A₃-A₄-A₅-A₆-A₇-A₈-A₉-A₁₀-A₁₁-A₁₂ (Formula 2)

where A₁ to A₁₂ each independently represent an amino acid;

X is not present, or is a moiety consisting of 1 to 9 amino acids in length;

Helix B is a moiety which consists of 11 to 13 amino acids in length and can form an α -helix structure;

L is a moiety consisting of 2 to 7 amino acids in length and represented by Formula 3:

[Formula 3]



where the amino acids are numbered from the C-terminal as "i" (-1), "ii" (-2), ... and

L_{iii} to L_{vii} may not be present, and

a combination of three amino acids A_1 , A_4 , and L_{ii} or a combination of two amino acids A_4 and L_{ii} corresponds to a base or base sequence of the target mRNA,

wherein one or more functional domains (A) are selected from the group consisting of a domain which guides ribosome to the mRNA, a domain which initiates or promotes translation of the mRNA, a domain associated with nuclear export of the mRNA, a domain which binds to an endoplasmic reticulum membrane, a domain containing an endoplasmic reticulum retention signal (ER retention signal) sequence, and a domain containing an endoplasmic reticulum signal sequence.

[Claim 2]

The fusion protein according to claim 1, wherein polypeptide moiety (B) comprises 2 to 30 PPR motifs, and the plurality of PPR motifs is arranged so as to specifically bind to the base sequence of the target mRNA.

[Claim 3]

The fusion protein according to claim 2, polypeptide moiety (B) comprises 5 to 25 PPR motifs.

[Claim 4]

The fusion protein according to any one of claims 1 to 3, wherein one or more functional domains (A) each bind to an N-terminal and/or a C-terminal of polypeptide moiety (B).

[Claim 5]

The fusion protein according to any one of the preceding claims,

wherein the domain which guides ribosome to the mRNA is a domain containing all or functional part of a polypeptide selected from the group consisting of DENR (Density-regulated protein), MCT-1 (Malignant T-cell amplified sequence 1), TPT1 (Translationally-controlled tumor protein), and Lerepo4 (Zinc finger CCCH-domain),

the domain initiating or promoting translation of the mRNA is a domain containing all or functional part of a polypeptide selected from the group consisting of eIF4E and eIF4G,

the domain associated with nuclear export of the mRNA is a domain containing all or functional part of SLBP (Stem-loop binding protein),

the domain binding to an endoplasmic reticulum membrane is a domain containing all or functional part of a polypeptide selected from the group consisting of SEC61B, TRAP-alpha (Translocon associated protein alpha), SR-alpha, Dia1 (Cytochrome b5 reductase 3), and p180,

the endoplasmic reticulum retention signal (ER retention signal) sequence is a signal sequence containing a KDEL (KEEL) sequence, or

the endoplasmic reticulum signal sequence is a signal sequence containing MGWSCIILFLVATATGAHS.

[Claim 6]

The fusion protein according to any one of claims 1 to 5, wherein the combination of the three amino acids A_1 , A_4 , and L_{ii} in each of the PPR motifs is:

(valine, threonine, asparagine), (phenylalanine, serine, asparagine), (phenylalanine, threonine, asparagine), (isoleucine, asparagine, aspartic acid), or (threonine, threonine, asparagine) in order of (A_1 , A_4 , L_{ii}) if a target base for the PPR motif is A (adenine);

(glutamic acid, glycine, aspartic acid), (valine, threonine, aspartic acid), (lysine, threonine, aspartic acid), or (leucine, threonine, aspartic acid) in order of (A_1 , A_4 , L_{ii}) if the target base for the PPR motif is G (guanine);

(valine, asparagine, aspartic acid), (isoleucine, asparagine, asparagine), (isoleucine, asparagine, aspartic acid), (isoleucine, methionine, aspartic acid), (phenylalanine, proline, aspartic acid), or (tyrosine, proline, aspartic acid) in order of (A_1 , A_4 , L_{ii}) if the target base for the PPR motif is U (uracil); or

(valine, asparagine, asparagine), (isoleucine, asparagine, asparagine), (valine, asparagine, serine), or (isoleucine, methionine, aspartic acid) in order of (A_1 , A_4 , L_{ii}) if the target base for the PPR motif is C (cytosine).

[Claim 7]

The fusion protein according to any one of claims 1 to 5, wherein the combination of the two amino acids A_4 and L_{ii} in each of the PPR motifs is:

(threonine, asparagine), (serine, asparagine), or (glycine, asparagine) in order of (A_4 , L_{ii}) if a target base for the PPR motif is A (adenine);

(threonine, aspartic acid) or (glycine, aspartic acid) in order of (A_4 , L_{ii}) if the target base for the PPR motif is G (guanine);

(asparagine, aspartic acid), (proline, aspartic acid), (methionine, aspartic acid), or (valine, threonine) in order of (A_4 , L_{ii}) if the target base for the PPR motif is U (uracil); or

(asparagine, asparagine), (asparagine, serine), or (leucine, aspartic acid) in order of (A_4 , L_{ii}) if the target base for the PPR motif is C (cytosine).

[Claim 8]

A nucleic acid encoding the fusion protein according to any one of claims 1 to 7.

[Claim 9]

A vector comprising the nucleic acid according to claim 8.

[Claim 10]

The vector according to claim 9, wherein the vector is an expression vector.

[Claim 11]

An in vitro method of improving a protein expression level from a target mRNA within a cell, the method comprising:

a step of providing the fusion protein according to any one of claims 1 to 8 or the vector according to claim 9 or 10; and

a step of introducing the fusion protein or the vector into a cell.

[Claim 12]

The method according to claim 11,
wherein the cell is a eukaryotic cell.

[Claim 13]

The method according to claim 12,
wherein the cell is an animal cell.

[Claim 14]

The method according to claim 13,
wherein the animal cell is a human cell.