TREM COMPOSITIONS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application 62/794,342 filed on January 18, 2019, and U.S. Provisional Application 62/855,547 filed on May 31, 2019, the entire contents of each of which are hereby incorporated by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on January 8, 2020, is named F2099-7000WO_SL.txt and is 228,808 bytes in size.

BACKGROUND

tRNAs are complex RNA molecules that possess a number of functions including the initiation and elongation of proteins.

SUMMARY

In an aspect, the disclosure provides a method of making a purified tRNA effector molecule (TREM) pharmaceutical composition, comprising:

providing a mammalian host cell comprising an exogenous nucleic acid, e.g., a DNA or RNA, encoding the TREM;

maintaining the mammalian cell under conditions sufficient to express the TREM;

purifying the TREM from the mammalian host cell, e.g., according to a method described herein; and

formulating the purified TREM as a pharmaceutical composition, e.g., by combining the TREM with a pharmaceutical excipient,

thereby making the TREM pharmaceutical composition.

In an example, the nucleic acid comprises an RNA, which upon reverse transcription, results in a DNA which can be transcribed into the TREM.

In an example, the nucleic acid comprises an RNA sequence at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%) identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof.

In an example, the nucleic acid comprises an RNA sequence comprising a consensus sequence, e.g., as provided herein, e.g., a consensus sequence of Formula I $_{ZZZ}$, Formula II $_{ZZZ}$, or Formula III $_{ZZZ}$, wherein $_{ZZZ}$ indicates any of the twenty amino acids:Alanine, Arginine, Asparagine, Aspartate, Cysteine, Glutamine, Glutamate, Glycine, Histidine, Isoleucine, Methionine, Leucine, Lysine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, or Valine.

In an example, the mammalian host cell is chosen from: a non-human cell or cell line, or a human cell or cell line, e.g., a HEK293T cell (*e.g.*, a Freestyle 293-F cell), a HT-1080 cell, a PER.C6 cell, a HKB-11 cell, a CAP cell, a HuH-7 cell, a BHK 21 cell, an MRC-S cell, a MDCK cell, a VERO cell, a WI-38 cell, a Chinese Hamster Ovary (CHO) cell, or a MCF7 cell.

In an example, the purification step comprises one, two or all of the following steps, e.g., in the order recited:

(i) separating nucleic acids from cellular debris to provide an RNA preparation;

(ii) separating RNA of less than a threshold number of nucleotides, e.g., less than 500 nt, less than 400 nt, less than 300 nt, less than 250 nt, less than 200nt, less than 150 nt, from larger RNA species in the RNA preparation to produce a small RNA preparation; or/and

(iii) separating a TREM from other RNA species in the small RNA preparation by affinity-based separation, e.g., sequence affinity-based separation.

In another aspect, there is provided a composition comprising a purified tRNA effector molecule (TREM) (e.g., a purified TREM composition made according to a method described herein), comprising:

(i) an RNA sequence at least 90% identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof; or

(ii) an RNA sequence comprising a consensus sequence provided herein.

In another aspect, there is provided a GMP-grade, recombinant TREM composition (*e.g.*, a TREM composition made in compliance with cGMP, and/or in accordance with similar requirements) comprising:

(i) an RNA sequence at least 90% identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof; or

(ii) an RNA sequence comprising a consensus sequence provided herein.

In one aspect, the disclosure features a method of making a tRNA effector molecule (TREM) composition, comprising:

(a) providing a host cell, comprising exogenous nucleic acid, *e.g.*, a DNA or RNA, encoding a TREM under conditions sufficient to express the TREM, and

(b) purifying the expressed TREM from the host cell culture to produce a TREM composition,

thereby making a TREM composition.

In an example, the TREM composition is a pharmaceutically acceptable composition.

In another example, the disclosure features a method of making a pharmaceutical TREM composition, comprising:

a) providing a purified TREM composition, *e.g.*, a purified TREM composition made by culturing a mammalian host cell comprising DNA or RNA encoding a TREM under conditions sufficient to express the TREM, and purifying the expressed TREM from the host cell culture to produce a purified TREM composition,

b) providing a value, *e.g.*, by evaluating or testing, for a characteristic described herein (e.g., a characteristic related to identity (e.g., sequence), purity (e.g., process impurity such as TREM fragments, host cell protein or host cell DNA), activity (e.g., adaptor activity)),

c) optionally, formulating the purified TREM composition as a pharmaceutical drug product (*e.g.*, combining the TREM composition with a pharmaceutical excipient) if it meets a reference criterion for the one or more characteristic,

thereby making the pharmaceutical TREM composition.

In another aspect, the disclosure features a method of making a pharmaceutical TREM composition comprising:

combining

a) a TREM, *e.g.*, a purified TREM composition, *e.g.*, a TREM composition made by a method described herein; and

b) a pharmaceutically acceptable component, *e.g.*, an excipient,

thereby making a pharmaceutical TREM composition.

In another aspect, there is provided a method of making a purified tRNA effector molecule (TREM) pharmaceutical composition, comprising:

purifying the TREM from a mammalian host cell;

formulating the purified TREM as a pharmaceutical composition, e.g., by combining the TREM with a pharmaceutical excipient,

thereby making the TREM pharmaceutical composition.

In another aspect, there is provided a method of making a TREM composition, comprising:

contacting a TREM containing a reaction mixture with a reagent, *e.g.*, a capture reagent or a separation reagent, comprising a nucleic acid sequence complimentary with a TREM;

thereby making a TREM composition.

In another aspect, there is provided a a method of making a pharmaceutical composition, comprising:

a) providing a purified TREM composition, *e.g.*, a purified TREM composition made by culturing a mammalian host cell comprising DNA or RNA encoding a TREM under conditions sufficient to express the TREM, and purifying the expressed TREM from the host cell culture to produce a purified TREM composition,

b) providing a value, *e.g.*, by evaluating or testing, for one or more of the following characteristics of the purified TREM composition:

(i) purity of at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%;

(ii) host cell protein (HCP) contamination of less than 0.1ng/ml, 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(iii) host cell protein (HCP) contamination of less than 0.1ng, 1ng, 5ng, 10ng, 15ng, 20ng, 25ng, 30ng, 35ng, 40ng, 50ng, 60ng, 70ng, 80ng, 90ng, or 100ng per milligram (mg) of the TREM composition;

(iv) DNA, *e.g.*, host cell DNA, of less than 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(v) less than 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% TREM fragments relative to full length TREMs;

(vi) low levels or absence of endotoxins, *e.g.*, a negative result as measured by the Limulus amebocyte lysate (LAL) test;

(vii) in-vitro translation activity, *e.g.*, as measured by an assay described in Example 15;

(viii) TREM concentration of at least 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL, 0.1 ug/mL, 0.5 ug/mL,1 ug/mL, 2 ug/mL, 5 ug/mL, 10 ug/mL, 20

ug/mL, 30 ug/mL, 40 ug/mL, 50 ug/mL, 60 ug/mL, 70 ug/mL, 80 ug/mL, 100 ug/mL, 200 ug/mL, 300 ug/mL, 500 ug/mL, 1000 ug/mL, 5000 ug/mL, 10,000 ug/mL, or 100,000 ug/mL;

(ix) sterility, *e.g.*, as per cGMP guidelines for sterile drug products, *e.g.*, the composition or preparation supports the growth of fewer than 100 viable microorganisms as tested under aseptic conditions, the composition or preparation meets the standard of USP <71>, and/or the composition or preparation meets the standard of USP <85>; or

(x) viral contamination, *e.g.*, the composition or preparation has an absence of, or an undetectable level of viral contamination.

c) optionally, formulating the purified TREM composition as a pharmaceutical drug product (*e.g.*, combining the TREM composition with a pharmaceutical excipient) if it meets a reference criteria for the one or more characteristics,

thereby making a pharmaceutical composition.

In another aspect, there is provided a pharmaceutical tRNA effector molecule (TREM) composition, comprising

(i) an RNA sequence at least 80% identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof; or

(ii) an RNA sequence comprising a consensus sequence provided herein.

In another aspect, there is provided a recombinant TREM composition of at least 0.5g, 1g, 2g, 3g, 4 g, 5g, 6g, 7g, 8g, 9g, 10g, 15g, 20g, 30g, 40g, 50g, 100g, 200g, 300g, 400g or 500g.

In another aspect, there is provided a recombinant TREM composition of between 0.5g to 500g, between 0.5g to 400g, between 0.5g to 300g, between 0.5g to 200g, between 0.5g to 100g, between 0.5g to 50g, between 0.5g to 40g, between 0.5g to 30g, between 0.5g to 20g, between 0.5g to 10g, between 0.5g to 9g, between 0.5g to 8g, between 0.5g to 7g, between 0.5g to 6g, between 0.5g to 5g, between 0.5g to 4g, between 0.5g to 3g, between 0.5g to 2g, between 0.5g to 1g, between 1g to 500g, between 2g to 500g, between 5g to 500g, between 10g to 500g, between 20g to 500g, between 30g to 500g, between 40g to 500g, between 50g to 500g, between 100g to 500g, between 200g to 500g, between 300g to 500g, or between 400g to 500g.

In another example, the present disclosure provides a composition comprising a purified tRNA effector molecule (TREM) (e.g., a purified TREM composition made according to a method described herein), comprising an RNA sequence at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%) identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof.

In an example, the present disclosure provides a composition comprising a purified tRNA effector molecule (TREM) (e.g., a purified TREM composition made according to a method

described herein), comprising an RNA sequence comprising a consensus sequence provided herein, e.g., a consensus sequence of Formula I zzz, Formula II zzz, or Formula III zzz, wherein zzz indicates any of the twenty amino acids: Alanine, Arginine, Asparagine, Aspartate, Cysteine, Glutamine, Glutamate, Glycine, Histidine, Isoleucine, Methionine, Leucine, Lysine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, or Valine.

In another example, the disclosure features a GMP-grade, recombinant TREM composition (*e.g.*, a TREM composition made in compliance with cGMP, and/or in accordance with similar requirements) comprising an RNA sequence at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%) identical to an RNA encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof.

In another example, the disclosure features a GMP-grade, recombinant TREM composition (*e.g.*, a TREM composition made in compliance with cGMP, and/or in accordance with similar requirements) comprising an RNA sequence comprising a consensus sequence provided herein.

In an example, the disclosure features a TREM comprising a consensus sequence provided herein.

In an example, the disclosure features a TREM comprising a consensus sequence of Formula I zzz, wherein zzz indicates any of the twenty amino acids and Formula I corresponds to all species.

In an example, the disclosure features a TREM comprising a consensus sequence of Formula II zzz, wherein zzz indicates any of the twenty amino acids and Formula II corresponds to mammals.

In an example, the disclosure features a TREM comprising a consensus sequence of Formula III zzz, wherein zzz indicates any of the twenty amino acids and Formula III corresponds to humans.

In an example, ZZZ indicates any of the amino acids: Alanine, Arginine, Asparagine, Aspartate, Cysteine, Glutamine, Glutamate, Glycine, Histidine, Isoleucine, Methionine, Leucine, Lysine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, or Valine.

In another aspect, there is provided a TREM composition comprising a consensus sequence of Formula I ZZZ,

R_0- R_1- R_2- R_3-R_4 -R_5-R_6-R_7-R_8-R_9-R_{10}-R_{11}-R_{12}-R_{13}-R_{14}-R_{15}-R_{16}-R_{17}-R_{18}-R_{19}-R_{20}-R_{21}-R_{22}-R_{23}-R_{24}-R_{25}-R_{26}-R_{27}-R_{28}-R_{29}-R_{30}-R_{31}-R_{32}-R_{33}-R_{34}-R_{35}-R_{36}-R_{37}-R_{38}-R_{39}-R_{40}-R_{41}-R_{42}- R_{43}- R_{44}-R_{45}-R_{46}- $[R_{47}]_x$-R_{48}-R_{49}-R_{50}-R_{51}-R_{52}-R_{53}-R_{54}-R_{55}-R_{56}-R_{57}-R_{58}-R_{59}-R_{60}-R_{61}-R_{62}-R_{63}-R_{64}-R_{65}-R_{66}-R_{67}-R_{68}-R_{69}-R_{70}-R_{71}-R_{72}

wherein:

R is a ribonucleotide residue;

(i) ZZZ indicates any of the twenty amino acids;

(ii) Formula I corresponds to all species; and

(iii) x=1-271 (*e.g.*, x=1-250, x=1-225, x=1-200, x=1-175, x=1-150, x=1-125, x=1-100, x=1-75, x=1-50, x=1-40, x=1-30, x=1-29, x=1-28, x=1-27, x=1-26, x=1-25, x=1-24, x=1-23, x=1-22, x=1-21, x=1-20, x=1-19, x=1-18, x=1-17, x=1-16, x=1-15, x=1-14, x=1-13, x=1-12, x=1-11, x=1-10, x=10-271, x=20-271, x=30-271, x=40-271, x=50-271, x=60-271, x=70-271, x=80-271, x=100-271, x=125-271, x=150-271, x=175-271, x=200-271, x=225-271, x=1, x=2, x=3, x=4, x=5, x=6, x=7, x=8, x=9, x=10, x=11, x=12, x=13, x=14, x=15, x=16, x=17, x=18, x=19, x=20, x=21, x=22, x=23, x=24, x=25, x=26, x=27, x=28, x=29, x=30, x=40, x=50, x=60, x=70, x=80, x=90, x=100, x=110, x=125, x=150, x=175, x=200, x=225, x=250, or x=271).

In another aspect, there is provided a TREM composition comprising a consensus sequence of Formula II ZZZ,

R_0- R_1-R_2- R_3-R_4 -R_5-R_6-R_7-R_8-R_9-R_{10}-R_{11}-R_{12}-R_{13}-R_{14}-R_{15}-R_{16}-R_{17}-R_{18}-R_{19}-R_{20}-R_{21}-R_{22}-R_{23}-R_{24}-R_{25}-R_{26}-R_{27}-R_{28}-R_{29}-R_{30}-R_{31}-R_{32}-R_{33}-R_{34}-R_{35}-R_{36}-R_{37}-R_{38}-R_{39}-R_{40}-R_{41}-R_{42}- R_{43}- R_{44}-R_{45}-R_{46}- $[R_{47}]_x$-R_{48}-R_{49}-R_{50}-R_{51}-R_{52}-R_{53}-R_{54}-R_{55}-R_{56}-R_{57}-R_{58}-R_{59}-R_{60}-R_{61}-R_{62}-R_{63}-R_{64}-R_{65}-R_{66}-R_{67}-R_{68}-R_{69}-R_{70}-R_{71}-R_{72}

wherein:

R is a ribonucleotide residue;

(i) ZZZ indicates any of the twenty amino acids;

(ii) Formula II corresponds to mammals; and

(iii) x=1-271 (*e.g.*, x=1-250, x=1-225, x=1-200, x=1-175, x=1-150, x=1-125, x=1-100, x=1-75, x=1-50, x=1-40, x=1-30, x=1-29, x=1-28, x=1-27, x=1-26, x=1-25, x=1-24, x=1-23, x=1-22, x=1-21, x=1-20, x=1-19, x=1-18, x=1-17, x=1-16, x=1-15, x=1-14, x=1-13, x=1-12, x=1-11, x=1-10, x=10-271, x=20-271, x=30-271, x=40-271, x=50-271, x=60-271, x=70-271, x=80-271, x=100-271, x=125-271, x=150-271, x=175-271, x=200-271, x=225-271, x=1, x=2, x=3, x=4, x=5, x=6, x=7, x=8, x=9, x=10, x=11, x=12, x=13, x=14, x=15, x=16, x=17, x=18, x=19, x=20, x=21, x=22, x=23, x=24, x=25, x=26, x=27, x=28, x=29, x=30, x=40, x=50, x=60, x=70, x=80, x=90, x=100, x=110, x=125, x=150, x=175, x=200, x=225, x=250, or x=271).

In another aspect, there is provided a TREM composition comprising a consensus sequence of Formula III ZZZ,

R_0- R_1-R_2- R_3-R_4 -R_5-R_6-R_7-R_8-R_9-R_{10}-R_{11}-R_{12}-R_{13}-R_{14}-R_{15}-R_{16}-R_{17}-R_{18}-R_{19}-R_{20}-R_{21}-R_{22}-R_{23}-R_{24}-R_{25}-R_{26}-R_{27}-R_{28}-R_{29}-R_{30}-R_{31}-R_{32}-R_{33}-R_{34}-R_{35}-R_{36}-R_{37}-R_{38}-R_{39}-R_{40}-R_{41}-R_{42}- R_{43}- R_{44}-R_{45}-R_{46}- $[R_{47}]_x$-R_{48}-R_{49}-R_{50}-R_{51}-R_{52}-R_{53}-R_{54}-R_{55}-R_{56}-R_{57}-R_{58}-R_{59}-R_{60}-R_{61}-R_{62}-R_{63}-R_{64}-R_{65}-R_{66}-R_{67}-R_{68}-R_{69}-R_{70}-R_{71}-R_{72}

wherein:

R is a ribonucleotide residue;

(i) ZZZ indicates any of the twenty amino acids;

(ii) Formula III corresponds to humans; and

(iii) x=1-271 (*e.g.*, x=1-250, x=1-225, x=1-200, x=1-175, x=1-150, x=1-125, x=1-100, x=1-75, x=1-50, x=1-40, x=1-30, x=1-29, x=1-28, x=1-27, x=1-26, x=1-25, x=1-24, x=1-23, x=1-22, x=1-21, x=1-20, x=1-19, x=1-18, x=1-17, x=1-16, x=1-15, x=1-14, x=1-13, x=1-12, x=1-11, x=1-10, x=10-271, x=20-271, x=30-271, x=40-271, x=50-271, x=60-271, x=70-271, x=80-271, x=100-271, x=125-271, x=150-271, x=175-271, x=200-271, x=225-271, x=1, x=2, x=3, x=4, x=5, x=6, x=7, x=8, x=9, x=10, x=11, x=12, x=13, x=14, x=15, x=16, x=17, x=18, x=19, x=20, x=21, x=22, x=23, x=24, x=25, x=26, x=27, x=28, x=29, x=30, x=40, x=50, x=60, x=70, x=80, x=90, x=100, x=110, x=125, x=150, x=175, x=200, x=225, x=250, or x=271).

In an example, the disclosure features a GMP-grade, recombinant TREM composition comprising an RNA sequence comprising a consensus sequence provided herein.

In an example of any of the TREM compositions or pharmaceutical TREM compositions provided herein, the composition comprises one or more, *e.g.*, a plurality, of TREMs.

In an example of any of the TREM compositions or pharmaceutical TREM compositions provided herein, the composition comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 species of TREMs.

In an example of any of the TREM compositions or pharmaceutical TREM compositions provided herein, the TREM composition (or an intermediate in the production of a TREM composition) comprises one or more of the following characteristics:

(i) purity of at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%;

(ii) host cell protein (HCP) contamination of less than 0.1ng/ml, 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(iii) host cell protein (HCP) contamination of less than 0.1ng, 1ng, 5ng, 10ng, 15ng, 20ng, 25ng, 30ng, 35ng, 40ng, 50ng, 60ng, 70ng, 80ng, 90ng, or 100ng, per milligram (mg) of the TREM composition;

(iv) DNA, *e.g.*, host cell DNA, of less than 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(v) Fragments of less than 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10%;

(vi) low levels or absence of endotoxins, *e.g.*, a negative result as measured by the Limulus amebocyte lysate (LAL) test;

(vii) in-vitro translation activity, *e.g.*, as measured by an assay described in Example 15;

(viii) TREM concentration of at least 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL, 0.1 ug/mL, 0.5 ug/mL,1 ug/mL, 2 ug/mL, 5 ug/mL, 10 ug/mL, 20 ug/mL, 30 ug/mL, 40 ug/mL, 50 ug/mL, 60 ug/mL, 70 ug/mL, 80 ug/mL, 100 ug/mL,

200 ug/mL, 300 ug/mL, 500 ug/mL, 1000 ug/mL, 5000 ug/mL, 10,000 ug/mL, or 100,000 ug/mL;

(ix) sterility, *e.g.*, as per cGMP guidelines for sterile drug products, *e.g.*, the composition or preparation supports the growth of fewer than 100 viable microorganisms as tested under aseptic conditions, the composition or preparation meets the standard of USP <71>, and/or the composition or preparation meets the standard of USP <85>; or

(x) viral contamination, *e.g.*, the composition or preparation has an absence of, or an undetectable level of viral contamination.

In another aspect, there is provided a cell comprising a TREM made according to a method of making a TREM disclosed herein.

In another aspect, there is provided a cell comprising a TREM disclosed herein.

In another aspect, the disclosure features, a cell comprising an exogenous nucleic acid comprising:

a nucleic acid sequence, *e.g.*, DNA or RNA, that encodes a TREM, wherein the nucleic acid sequence comprises:

(i) a control region sequence;

(ii) a sequence encoding a modified TREM;

(iii) a sequence encoding more than one TREM;

(iv) a sequence other than a $tRNA^{Met}$ sequence; or

(v) a promoter sequence that comprises a Pol III recognition site, *e.g.*, a U6 promoter, a 7SK promoter or a H1 promoter, or a fragment thereof.

In another aspect, there is provided a method of contacting a cell, tissue, or subject with a TREM, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby contacting a cell, tissue, or subject with the TREM.

In another aspect, there is provided a method of presenting a TREM to a cell, tissue, or subject with a TREM, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby presenting the TREM to a cell, tissue, or subject.

In another aspect, there is provided a method of forming a TREM-contacted cell, tissue, or subject, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby forming a TREM-contacted cell, tissue, or subject.

In another aspect, there is provided a method of using a TREM comprising,

contacting the cell, tissue or subject with a purified TREM composition,

thereby using the TREM.

In another aspect, there is provided a method of applying a TREM to a cell, tissue, or subject, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby applying a TREM to a cell, tissue, or subject.

In another aspect, there is provided a method of exposing a cell, tissue, or subject to a TREM, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby exposing a cell, tissue, or subject to a TREM.

In another aspect, there is provided a method of forming an *admixture* of a TREM and a cell, tissue, or subject, comprising

contacting the cell, tissue or subject with a TREM composition,

thereby forming an admixture of a TREM and a cell, tissue, or subject.

In an aspect, the disclosure features a method of modulating a tRNA pool in a cell comprising:

providing a purified TREM composition, and contacting the cell with the TREM composition,

thereby modulating the tRNA pool in the cell.

In another aspect, the disclosure features a method of delivering a TREM to a cell, tissue, or subject, comprising:

providing a cell, tissue, or subject, and contacting the cell, tissue, or subject, with a TREM composition comprising the TREM, *e.g.*, a pharmaceutical TREM composition comprising the TREM.

In another aspect, there is provided a method, e.g., an ex vivo method, of modulating the metabolism, *e.g.*, the translational capacity of an organelle, comprising:

providing a preparation of an organelle, *e.g.*, mitochondria or chloroplasts, and contacting the organelle with a pharmaceutical TREM composition.

In another example, the disclosure features a method of treating a subject, *e.g.*, modulating the metabolism, *e.g.*, the translational capacity of a cell, in a subject, comprising:

providing, *e.g.*, administering to the subject, an exogenous nucleic acid, *e.g.*, a DNA or RNA, which encodes a TREM, thereby treating the subject.

In an aspect, there is provided the use of an exogenous nucleic acid, *e.g.*, a DNA or RNA, which encodes a TREM, in the manufacture of a medicament for the treatment e.g. modulating the metabolism, e.g. the translational capacity of a cell, of a subject.

In an example of any of the methods disclosed herein, the TREM composition is made by:

providing a mammalian host cell comprising an exogenous nucleic acid, e.g., a DNA or RNA, encoding the TREM;

maintaining the mammalian cell under conditions sufficient to express the TREM; and/or

purifying the TREM from the mammalian host cell, e.g., according to a method described herein.

In an example of any of the methods disclosed herein, the mammalian host cell is a non-human cell or cell line, or a human cell or cell line chosen from: a HEK293T cell (*e.g.*, a Freestyle 293-F cell), a HT-1080 cell, a PER.C6 cell, a HKB-11 cell, a CAP cell, a HuH-7 cell, a BHK 21 cell, an MRC-S cell, a MDCK cell, a VERO cell, a WI-38 cell, a Chinese Hamster Ovary (CHO) cell, or a MCF7 cell.

In an example of any of the methods disclosed herein, the purification step comprises one, two or all of the following steps, e.g., in the order recited:

(i) separating nucleic acids from cellular debris to provide an RNA preparation;

(ii) separating RNA of less than a threshold number of nucleotides, e.g., less than 500 nt, less than 400 nt, less than 300 nt, less than 250 nt, less than 200nt, less than 150 nt, from larger RNA species in the RNA preparation to produce a small RNA preparation; and/or

(iii) separating a TREM from other RNA species by affinity-based separation, e.g., sequence affinity-based separation.

In an example of any of the methods disclosed herein, the TREM comprises:

(i) an RNA sequence at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%) identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof; or

(ii) an RNA sequence comprising a consensus sequence provided herein.

In an example, the disclosure provides a method of making a purified tRNA effector molecule (TREM) pharmaceutical composition, comprising:

providing an insect host cell comprising an exogenous nucleic acid, e.g., a DNA or RNA, encoding the TREM;

maintaining the insect host cell under conditions sufficient to express the TREM;

purifying the TREM from the insect host cell, e.g., according to a method described herein; and

formulating the purified TREM as a pharmaceutical composition, e.g., by combining the TREM with a pharmaceutical excipient,

thereby making the TREM pharmaceutical composition.

In an example, the insect host cell is chosen from: an insect cell or cell line, e.g., a Sf9 cell or cell line.

In an example, the purification step comprises one, two or all of the following steps, e.g., in the order recited:

(i) separating nucleic acids from protein to provide an RNA preparation;

(ii) separating RNA of less than a threshold number of nucleotides, e.g., less than 500 nt, less than 400 nt, less than 300 nt, less than 250 nt, less than 200nt, less than 150 nt, from larger RNA species in the RNA preparation to produce a small RNA preparation; and/or

(iii) separating a TREM from other RNA species in the small RNA preparation by affinity-based separation, e.g., sequence affinity.

In an example, the disclosure provides a method of making a purified tRNA effector molecule (TREM) pharmaceutical composition, comprising:

providing a yeast host cell comprising an exogenous nucleic acid, e.g., a DNA or RNA, encoding the TREM;

maintaining the yeast host cell under conditions sufficient to express the TREM;

purifying the TREM from the yeast host cell, e.g., according to a method described herein; and

formulating the purified TREM as a pharmaceutical composition, e.g., by combining the TREM with a pharmaceutical excipient,

thereby making the TREM pharmaceutical composition.

In an example, the yeast host cell is chosen from: a yeast cell or cell line, e.g., a S. cerevisiae or S. pombe cell or cell line.

In an example, the purification step comprises one, two or all of the following steps, e.g., in the order recited:

(i) separating nucleic acids from protein to provide an RNA preparation;

(ii) separating RNA of less than a threshold number of nucleotides, e.g., less than 500 nt, less than 400 nt, less than 300 nt, less than 250 nt, less than 200nt, less than 150 nt, from larger RNA species in the RNA preparation to produce a small RNA preparation; and/or

(iii) separating a TREM from other RNA species in the small RNA preparation by affinity-based separation, e.g., sequence affinity.

In another aspect, there is provided a reaction mixture comprising a TREM and a reagent, *e.g.*, a capture reagent, or a separation reagent.

In another aspect, there is provided a bioreactor comprising a plurality of mammalian host cells described herein comprising exogenous DNA or RNA.

In another aspect, there is provided a master cell bank comprising a host cell, *e.g.*, as described herein.

In another aspect, there is provided a method of evaluating a composition of TREM, *e.g.*, a GMP-grade TREM (i.e., a TREM made in compliance with cGMP, and/or in accordance with similar requirements), comprising acquiring a value for one or more of the following characteristics of the purified TREM composition:

(i) purity of at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%;

(ii) host cell protein (HCP) contamination of less than 0.1ng/ml, 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(iii) host cell protein (HCP) contamination of less than 0.1ng, 1ng, 5ng, 10ng, 15ng, 20ng, 25ng, 30ng, 35ng, 40ng, 50ng, 60ng, 70ng, 80ng, 90ng, or 100ng per milligram (mg) of the TREM composition;

(iv) DNA, *e.g.*, host cell DNA, of less than 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(v) less than 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% TREM fragments relative to full length TREMs;

(vi) low levels or absence of endotoxins, *e.g.*, a negative result as measured by the Limulus amebocyte lysate (LAL) test;

(vii) in-vitro translation activity, *e.g.*, as measured by an assay described in Example 15;

(viii) TREM concentration of at least 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL, 0.1 ug/mL, 0.5 ug/mL,1 ug/mL, 2 ug/mL, 5 ug/mL, 10 ug/mL, 20 ug/mL, 30 ug/mL, 40 ug/mL, 50 ug/mL, 60 ug/mL, 70 ug/mL, 80 ug/mL, 100 ug/mL, 200 ug/mL, 300 ug/mL, 500 ug/mL, 1000 ug/mL, 5000 ug/mL, 10,000 ug/mL, or 100,000 ug/mL;

(ix) sterility, *e.g.*, , the composition or preparation supports the growth of fewer than 100 viable microorganisms as tested under aseptic conditions, the composition or preparation meets the standard of USP <71>, and/or the composition or preparation meets the standard of USP <85> as described by cGMP guidelines for sterile drug products produced by aseptic processing; or

(x) viral contamination, *e.g.*, the composition or preparation has an absence of, or an undetectable level of viral contamination.

As disclosed herein tRNA-based effector molecules (TREMs) are complex molecules which can mediate a variety of cellular processes. Pharmaceutical TREM compositions can be administered to cells, tissues or subjects to modulate these functions, *e.g.*, *in vitro* or *in vivo*. Disclosed herein are TREM compositions, preparations, methods of making TREM compositions and preparations, and methods of using TREM compositions and preparations.

Additional features of any of the aforesaid TREM compositions, preparations, methods of making TREM compositions and preparations, and methods of using TREM compositions and preparations include one or more of the following enumerated examples.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific examples of the invention described herein. Such equivalents are intended to be encompassed by the following enumerated examples.

What is claimed is:

1. A method of making a purified tRNA effector molecule (TREM) pharmaceutical composition, comprising:

providing a mammalian host cell comprising an exogenous nucleic acid, e.g., a DNA or RNA, encoding the TREM;

maintaining the mammalian cell under conditions sufficient to express the TREM;

purifying the TREM from the mammalian host cell, e.g., according to a method described herein; and

formulating the purified TREM as a pharmaceutical composition, e.g., by combining the TREM with a pharmaceutical excipient,

thereby making the TREM pharmaceutical composition.

2. The method of claim 1, wherein the nucleic acid comprises an RNA, which upon reverse transcription, results in a DNA which can be transcribed into the TREM.

3. The method of claim 1 or 2, wherein the nucleic acid comprises an RNA sequence at least 90% identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof.

4. The method of claim 1 or 2, wherein the nucleic acid comprises an RNA sequence comprising a consensus sequence provided herein.

5. The method of any one of the preceding claims, wherein the mammalian host cell is chosen from: a non-human cell or cell line, or a human cell or cell line, e.g., a HEK293T cell (*e.g.*, a Freestyle 293-F cell), a HT-1080 cell, a PER.C6 cell, a HKB-11 cell, a CAP cell, a HuH-7 cell, a BHK 21 cell, an MRC-S cell, a MDCK cell, a VERO cell, a WI-38 cell, a Chinese Hamster Ovary (CHO) cell, or a MCF7 cell.

6. The method of any one of the preceding claims, wherein the purification step comprises one, two or all of the following steps, e.g., in the order recited:

(i) separating nucleic acids from cellular debris to provide an RNA preparation;

(ii) separating RNA of less than a threshold number of nucleotides, e.g., less than 500 nt, less than 400 nt, less than 300 nt, less than 250 nt, less than 200nt, less than 150 nt, from larger RNA species in the RNA preparation to produce a small RNA preparation; and/or

(iii) separating a TREM from other RNA species in the small RNA preparation by affinity-based separation, e.g., sequence affinity-based separation.

7. A composition comprising a purified tRNA effector molecule (TREM) (e.g., a purified TREM composition made according to a method described herein), comprising:

(i) an RNA sequence at least 90% identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof; or

(ii) an RNA sequence comprising a consensus sequence provided herein.

8. A GMP-grade, recombinant TREM composition (*e.g.*, a TREM composition made in compliance with cGMP, and/or in accordance with similar requirements) comprising:

(i) an RNA sequence at least 90% identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof; or

(ii) an RNA sequence comprising a consensus sequence provided herein.

9. The TREM composition of claim 7 or 8, wherein the composition comprises one or more, *e.g.*, a plurality, of TREMs.

10. The TREM composition of any one of claims 7 to 9, wherein the composition comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 species of TREMs.

11. The TREM composition of any one of claims 7 to 10, wherein the TREM composition (or an intermediate in the production of a TREM composition) comprises one or more of the following characteristics:

(i) purity of at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%;

(ii) host cell protein (HCP) contamination of less than 0.1ng/ml, 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(iii) host cell protein (HCP) contamination of less than 0.1ng, 1ng, 5ng, 10ng, 15ng, 20ng, 25ng, 30ng, 35ng, 40ng, 50ng, 60ng, 70ng, 80ng, 90ng, or 100ng, per milligram (mg) of the TREM composition;

(iv) DNA, *e.g.*, host cell DNA, of less than 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(v) less than 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% TREM fragments relative to full length TREMs;

(vi) low levels or absence of endotoxins, *e.g.*, a negative result as measured by the Limulus amebocyte lysate (LAL) test;

(vii) in-vitro translation activity, *e.g.*, as measured by an assay described in Example 15;

(viii) TREM concentration of at least 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL, 0.1 ug/mL, 0.5 ug/mL,1 ug/mL, 2 ug/mL, 5 ug/mL, 10 ug/mL, 20 ug/mL, 30 ug/mL, 40 ug/mL, 50 ug/mL, 60 ug/mL, 70 ug/mL, 80 ug/mL, 100 ug/mL, 200 ug/mL, 300 ug/mL, 500 ug/mL, 1000 ug/mL, 5000 ug/mL, 10,000 ug/mL, or 100,000 ug/mL;

(ix) sterility, *e.g.*, as per cGMP guidelines for sterile drug products, *e.g.*, the composition or preparation supports the growth of fewer than 100 viable microorganisms as tested under aseptic conditions, the composition or preparation meets the standard of USP <71>, and/or the composition or preparation meets the standard of USP <85>; or

(x) viral contamination, *e.g.*, the composition or preparation has an absence of, or an undetectable level of viral contamination.

12. A method of modulating a tRNA pool in a cell comprising:

providing a purified TREM composition, and contacting the cell with the TREM composition,

thereby modulating the tRNA pool in the cell.

13. The method of claim 12, wherein the TREM composition is made by:

providing a mammalian host cell comprising an exogenous nucleic acid, e.g., a DNA or RNA, encoding the TREM;

maintaining the mammalian cell under conditions sufficient to express the TREM; and/or

purifying the TREM from the mammalian host cell, e.g., according to a method described herein.

14. The method of claim 12 or 13, wherein the mammalian host cell is chosen from: a non-human cell or cell line, or a human cell or cell line, e.g., a HEK293T cell (*e.g.*, a Freestyle 293-F cell), a HT-1080 cell, a PER.C6 cell, a HKB-11 cell, a CAP cell, a HuH-7 cell, a BHK 21 cell, an MRC-S cell, a MDCK cell, a VERO cell, a WI-38 cell, a Chinese Hamster Ovary (CHO) cell, or a MCF7 cell.

15. The method of any one of claims 12 to 14, wherein the purification step comprises one, two or all of the following steps, e.g., in the order recited:

(i) separating nucleic acids from cellular debris to provide an RNA preparation;

(ii) separating RNA of less than a threshold number of nucleotides, e.g., less than 500 nt, less than 400 nt, less than 300 nt, less than 250 nt, less than 200nt, less than 150 nt, from larger RNA species in the RNA preparation to produce a small RNA preparation; and/or

(iii) separating a TREM from other RNA species in the small RNA preparation by affinity-based separation, e.g., sequence affinity-based separation.

16. The method of any one of claims 12 to 15, wherein the TREM comprises:

(i) an RNA sequence at least 80% identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof; or

(ii) an RNA sequence comprising a consensus sequence provided herein.

17. A method of making a tRNA effector molecule (TREM) composition, comprising:

(a) providing a mammalian host cell comprising exogenous nucleic acid, *e.g.*, a DNA or RNA, encoding a TREM under conditions sufficient to express the TREM, and

(b) purifying the expressed TREM from the mammalian host cell to produce a TREM composition,

thereby making the TREM composition.

18. A method of making a pharmaceutical TREM composition comprising:

combining

a) a TREM, *e.g.*, a purified TREM composition, *e.g.*, a TREM composition made by a method described herein; and

b) a pharmaceutically acceptable component, *e.g.*, an excipient,

thereby making a pharmaceutical TREM composition.

19. A method of making a purified tRNA effector molecule (TREM) pharmaceutical composition, comprising:

purifying the TREM from a mammalian host cell;

formulating the purified TREM as a pharmaceutical composition, e.g., by combining the TREM with a pharmaceutical excipient,

thereby making the TREM pharmaceutical composition.

20. A method of making a TREM composition, comprising:

contacting a TREM containing a reaction mixture with a reagent, *e.g.*, a capture reagent or a separation reagent, comprising a nucleic acid sequence complimentary with a TREM;

thereby making a TREM composition.

21. A method of making a pharmaceutical composition, comprising:

a) providing a purified TREM composition, *e.g.*, a purified TREM composition made by culturing a mammalian host cell comprising DNA or RNA encoding a TREM under conditions sufficient to express the TREM, and purifying the expressed TREM from the host cell culture to produce a purified TREM composition,

b) providing a value, *e.g.*, by evaluating or testing, for one or more of the following characteristics of the purified TREM composition:

(i) purity of at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%;

(ii) host cell protein (HCP) contamination of less than 0.1ng/ml, 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(iii) host cell protein (HCP) contamination of less than 0.1ng, 1ng, 5ng, 10ng, 15ng, 20ng, 25ng, 30ng, 35ng, 40ng, 50ng, 60ng, 70ng, 80ng, 90ng, or 100ng per milligram (mg) of the TREM composition;

(iv) DNA, *e.g.*, host cell DNA, of less than 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(v) less than 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% TREM fragments relative to full length TREMs;

(vi) low levels or absence of endotoxins, *e.g.*, a negative result as measured by the Limulus amebocyte lysate (LAL) test;

(vii) in-vitro translation activity, *e.g.*, as measured by an assay described in Example 15;

(viii) TREM concentration of at least 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL, 0.1 ug/mL, 0.5 ug/mL,1 ug/mL, 2 ug/mL, 5 ug/mL, 10 ug/mL, 20 ug/mL, 30 ug/mL, 40 ug/mL, 50 ug/mL, 60 ug/mL, 70 ug/mL, 80 ug/mL, 100 ug/mL, 200 ug/mL, 300 ug/mL, 500 ug/mL, 1000 ug/mL, 5000 ug/mL, 10,000 ug/mL, or 100,000 ug/mL;

(ix) sterility, *e.g.*, as per cGMP guidelines for sterile drug products, *e.g.*, the composition or preparation supports the growth of fewer than 100 viable microorganisms as tested under aseptic conditions, the composition or preparation meets the standard of USP <71>, and/or the composition or preparation meets the standard of USP <85>; or

(x) viral contamination, *e.g.*, the composition or preparation has an absence of, or an undetectable level of viral contamination.

c) optionally, formulating the purified TREM composition as a pharmaceutical drug product (*e.g.*, combining the TREM composition with a pharmaceutical excipient) if it meets a reference criteria for the one or more characteristics,

thereby making a pharmaceutical composition.

22. A pharmaceutical tRNA effector molecule (TREM) composition, comprising

(i) an RNA sequence at least 80% identical to an RNA sequence encoded by a DNA sequence listed in Table 1, or a fragment or functional fragment thereof; or

(ii) an RNA sequence comprising a consensus sequence provided herein.

23. A recombinant TREM composition of at least 0.5g, 1g, 2g, 3g, 4 g, 5g, 6g, 7g, 8g, 9g, 10g, 15g, 20g, 30g, 40g, 50g, 100g, 200g, 300g, 400g or 500g.

24. A recombinant TREM composition of between 0.5g to 500g, between 0.5g to 400g, between 0.5g to 300g, between 0.5g to 200g, between 0.5g to 100g, between 0.5g to 50g, between 0.5g to 40g, between 0.5g to 30g, between 0.5g to 20g, between 0.5g to 10g, between 0.5g to 9g, between 0.5g to 8g, between 0.5g to 7g, between 0.5g to 6g, between 0.5g to 5g, between 0.5g to 4g, between 0.5g to 3g, between 0.5g to 2g, between 0.5g to 1g, between 1g to 500g, between 2g to 500g, between 5g to 500g, between 10g to 500g, between 20g to 500g, between 30g to 500g, between 40g to 500g, between 50g to 500g, between 100g to 500g, between 200g to 500g, between 300g to 500g, or between 400g to 500g.

25. A TREM composition comprising a consensus sequence of Formula I zzz,

R_0- R_{1-} R_2- R_3-R_4 -R_5-R_6-R_7-R_8-R_9-R_{10}-R_{11}-R_{12}-R_{13}-R_{14}-R_{15}-R_{16}-R_{17}-R_{18}-R_{19}-R_{20}-R_{21}-R_{22}-R_{23}-R_{24}-R_{25}-R_{26}-R_{27}-R_{28}-R_{29}-R_{30}-R_{31}-R_{32}-R_{33}-R_{34}-R_{35}-R_{36}-R_{37}-R_{38}-R_{39}-R_{40}-R_{41}-R_{42}- R_{43}- R_{44}-R_{45}-R_{46}- $[R_{47}]_x$-R_{48}-R_{49}-R_{50}-R_{51}-R_{52}-R_{53}-R_{54}-R_{55}-R_{56}-R_{57}-R_{58}-R_{59}-R_{60}-R_{61}-R_{62}-R_{63}-R_{64}-R_{65}-R_{66}-R_{67}-R_{68}-R_{69}-R_{70}-R_{71}-R_{72}

wherein:

R is a ribonucleotide residue;

(i) zzz indicates any of the twenty amino acids;

(ii) Formula I corresponds to all species; and

(iii) x=1-271 (*e.g.*, x=1-250, x=1-225, x=1-200, x=1-175, x=1-150, x=1-125, x=1-100, x=1-75, x=1-50, x=1-40, x=1-30, x=1-29, x=1-28, x=1-27, x=1-26, x=1-25, x=1-24, x=1-23, x=1-22, x=1-21, x=1-20, x=1-19, x=1-18, x=1-17, x=1-16, x=1-15, x=1-14, x=1-13, x=1-12, x=1-11, x=1-10, x=10-271, x=20-271, x=30-271, x=40-271, x=50-271, x=60-271, x=70-271,

x=80-271, x=100-271, x=125-271, x=150-271, x=175-271, x=200-271, x=225-271, x=1, x=2, x=3, x=4, x=5, x=6, x=7, x=8, x=9, x=10, x=11, x=12, x=13, x=14, x=15, x=16, x=17, x=18, x=19, x=20, x=21, x=22, x=23, x=24, x=25, x=26, x=27, x=28, x=29, x=30, x=40, x=50, x=60, x=70, x=80, x=90, x=100, x=110, x=125, x=150, x=175, x=200, x=225, x=250, or x=271).

26. A TREM composition comprising a consensus sequence of Formula II $_{ZZZ}$,

R_0- R_1-R_2- R_3-R_4 -R_5-R_6-R_7-R_8-R_9-R_{10}-R_{11}-R_{12}-R_{13}-R_{14}-R_{15}-R_{16}-R_{17}-R_{18}-R_{19}-R_{20}-R_{21}-R_{22}-R_{23}-R_{24}-R_{25}-R_{26}-R_{27}-R_{28}-R_{29}-R_{30}-R_{31}-R_{32}-R_{33}-R_{34}-R_{35}-R_{36}-R_{37}-R_{38}-R_{39}-R_{40}-R_{41}-R_{42}- R_{43}- R_{44}-R_{45}-R_{46}- $[R_{47}]_x$-R_{48}-R_{49}-R_{50}-R_{51}-R_{52}-R_{53}-R_{54}-R_{55}-R_{56}-R_{57}-R_{58}-R_{59}-R_{60}-R_{61}-R_{62}-R_{63}-R_{64}-R_{65}-R_{66}-R_{67}-R_{68}-R_{69}-R_{70}-R_{71}-R_{72}

wherein:

R is a ribonucleotide residue;

(i) $_{ZZZ}$ indicates any of the twenty amino acids;

(ii) Formula II corresponds to mammals; and

(iii) x=1-271 (*e.g.*, x=1-250, x=1-225, x=1-200, x=1-175, x=1-150, x=1-125, x=1-100, x=1-75, x=1-50, x=1-40, x=1-30, x=1-29, x=1-28, x=1-27, x=1-26, x=1-25, x=1-24, x=1-23, x=1-22, x=1-21, x=1-20, x=1-19, x=1-18, x=1-17, x=1-16, x=1-15, x=1-14, x=1-13, x=1-12, x=1-11, x=1-10, x=10-271, x=20-271, x=30-271, x=40-271, x=50-271, x=60-271, x=70-271, x=80-271, x=100-271, x=125-271, x=150-271, x=175-271, x=200-271, x=225-271, x=1, x=2, x=3, x=4, x=5, x=6, x=7, x=8, x=9, x=10, x=11, x=12, x=13, x=14, x=15, x=16, x=17, x=18, x=19, x=20, x=21, x=22, x=23, x=24, x=25, x=26, x=27, x=28, x=29, x=30, x=40, x=50, x=60, x=70, x=80, x=90, x=100, x=110, x=125, x=150, x=175, x=200, x=225, x=250, or x=271).

27. A TREM composition comprising a consensus sequence of Formula III $_{ZZZ}$,

R_0- R_1-R_2- R_3-R_4 -R_5-R_6-R_7-R_8-R_9-R_{10}-R_{11}-R_{12}-R_{13}-R_{14}-R_{15}-R_{16}-R_{17}-R_{18}-R_{19}-R_{20}-R_{21}-R_{22}-R_{23}-R_{24}-R_{25}-R_{26}-R_{27}-R_{28}-R_{29}-R_{30}-R_{31}-R_{32}-R_{33}-R_{34}-R_{35}-R_{36}-R_{37}-R_{38}-R_{39}-R_{40}-R_{41}-R_{42}- R_{43}- R_{44}-R_{45}-R_{46}- $[R_{47}]_x$-R_{48}-R_{49}-R_{50}-R_{51}-R_{52}-R_{53}-R_{54}-R_{55}-R_{56}-R_{57}-R_{58}-R_{59}-R_{60}-R_{61}-R_{62}-R_{63}-R_{64}-R_{65}-R_{66}-R_{67}-R_{68}-R_{69}-R_{70}-R_{71}-R_{72}

wherein:

R is a ribonucleotide residue;

(i) $_{ZZZ}$ indicates any of the twenty amino acids;

(ii) Formula III corresponds to humans; and

(iii) x=1-271 (*e.g.*, x=1-250, x=1-225, x=1-200, x=1-175, x=1-150, x=1-125, x=1-100, x=1-75, x=1-50, x=1-40, x=1-30, x=1-29, x=1-28, x=1-27, x=1-26, x=1-25, x=1-24, x=1-23, x=1-22, x=1-21, x=1-20, x=1-19, x=1-18, x=1-17, x=1-16, x=1-15, x=1-14, x=1-13, x=1-12, x=1-11, x=1-10, x=10-271, x=20-271, x=30-271, x=40-271, x=50-271, x=60-271, x=70-271, x=80-271, x=100-271, x=125-271, x=150-271, x=175-271, x=200-271, x=225-271, x=1, x=2, x=3, x=4, x=5, x=6, x=7, x=8, x=9, x=10, x=11, x=12, x=13, x=14, x=15, x=16, x=17, x=18, x=19, x=20, x=21, x=22, x=23, x=24, x=25, x=26, x=27, x=28, x=29, x=30, x=40, x=50, x=60, x=70, x=80, x=90, x=100, x=110, x=125, x=150, x=175, x=200, x=225, x=250, or x=271).

28. A method of contacting a cell, tissue, or subject with a TREM, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby contacting a cell, tissue, or subject with the TREM.

29. A method of presenting a TREM to a cell, tissue, or subject with a TREM, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby presenting the TREM to a cell, tissue, or subject.

30. A method of forming a TREM-contacted cell, tissue, or subject, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby forming a TREM-contacted cell, tissue, or subject.

31. A method of using a TREM comprising,

contacting the cell, tissue or subject with a purified TREM composition,

thereby using the TREM.

32. A method of applying a TREM to a cell, tissue, or subject, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby applying a TREM to a cell, tissue, or subject.

33. A method of exposing a cell, tissue, or subject to a TREM, comprising

contacting the cell, tissue or subject with a purified TREM composition,

thereby exposing a cell, tissue, or subject to a TREM.

34. A method of forming an *admixture* of a TREM and a cell, tissue, or subject, comprising

contacting the cell, tissue or subject with a TREM composition,

thereby forming an admixture of a TREM and a cell, tissue, or subject.

35. A method of delivering a TREM to a cell, tissue, or subject, comprising:

providing a cell, tissue, or subject, and contacting the cell, tissue, or subject, with a TREM composition, *e.g.*, a purified TREM composition, e.g., a pharmaceutical TREM composition.

36. A method, e.g., an ex vivo method, of modulating the metabolism, *e.g.*, the translational capacity of an organelle, comprising:

providing a preparation of an organelle, *e.g.*, mitochondria or chloroplasts, and contacting the organelle with a pharmaceutical TREM composition.

37. Use of an exogenous nucleic acid, *e.g.*, a DNA or RNA, which encodes a TREM, in the manufacture of a medicament for the treatment e.g. modulating the metabolism, e.g. the translational capacity of a cell, of a subject.

38. A cell comprising a TREM made according to a method of making a TREM disclosed herein.

39. A cell comprising a TREM disclosed herein.

40. A cell comprising an exogenous nucleic acid comprising:

a nucleic acid sequence, *e.g.*, DNA or RNA, that encodes a TREM, wherein the nucleic acid sequence comprises:

(i) a control region sequence;

(ii) a sequence encoding a modified TREM;

(iii) a sequence encoding more than one TREM;

(iv) a sequence other than a $tRNA^{Met}$ sequence; or

(v) a promoter sequence that comprises a Pol III recognition site, *e.g.*, a U6 promoter, a 7SK promoter or a H1 promoter, or a fragment thereof.

41. A reaction mixture comprising a TREM and a reagent, *e.g.*, a capture reagent, or a separation reagent.

42. A bioreactor comprising a plurality of mammalian host cells described herein comprising exogenous DNA or RNA.

43. A master cell bank comprising a host cell, *e.g.*, as described herein.

44. A method of evaluating a composition of TREM, *e.g.*, a GMP-grade TREM (i.e., a TREM made in compliance with cGMP, and/or in accordance with similar requirements), comprising acquiring a value for one or more of the following characteristics of the purified TREM composition:

(i) purity of at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%;

(ii) host cell protein (HCP) contamination of less than 0.1ng/ml, 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(iii) host cell protein (HCP) contamination of less than 0.1ng, 1ng, 5ng, 10ng, 15ng, 20ng, 25ng, 30ng, 35ng, 40ng, 50ng, 60ng, 70ng, 80ng, 90ng, or 100ng per milligram (mg) of the TREM composition;

(iv) DNA, *e.g.*, host cell DNA, of less than 1ng/ml, 5ng/ml, 10ng/ml, 15ng/ml, 20ng/ml, 25ng/ml, 30ng/ml, 35ng/ml, 40ng/ml, 50ng/ml, 60ng/ml, 70ng/ml, 80ng/ml, 90ng/ml, or 100ng/ml;

(v) less than 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% TREM fragments relative to full length TREMs;

(vi) low levels or absence of endotoxins, *e.g.*, a negative result as measured by the Limulus amebocyte lysate (LAL) test;

(vii) in-vitro translation activity, *e.g.*, as measured by an assay described in Example 15;
(viii) TREM concentration of at least 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL, 0.1 ug/mL, 0.5 ug/mL,1 ug/mL, 2 ug/mL, 5 ug/mL, 10 ug/mL, 20 ug/mL, 30 ug/mL, 40 ug/mL, 50 ug/mL, 60 ug/mL, 70 ug/mL, 80 ug/mL, 100 ug/mL, 200 ug/mL, 300 ug/mL, 500 ug/mL, 1000 ug/mL, 5000 ug/mL, 10,000 ug/mL, or 100,000 ug/mL;
(ix) sterility, *e.g.*, , the composition or preparation supports the growth of fewer than 100 viable microorganisms as tested under aseptic conditions, the composition or preparation meets the standard of USP <71>, and/or the composition or preparation meets the standard of USP <85> as described by cGMP guidelines for sterile drug products produced by aseptic processing; or
(x) viral contamination, *e.g.*, the composition or preparation has an absence of, or an undetectable level of viral contamination.