ribosome assembly, and function of higher eukaryotic rRNA have been hindered by the lack of an expression system that enables rRNA to be modified and then examined functionally.

[0006] There is a great need in the art for effective systems for expressing modified rRNAs and performing functional analyses, especially mammalian systems.

SUMMARY

[0007] In one aspect, there is provided a synthetic or isolated polynucleotide encoding a mammalian 18S rRNA that is resistant to pactamycin, wherein the pactamycin-resistance is conferred by one or more single residue substitutions in the 18S rRNA sequence, wherein the single residue substitution is at a position corresponding to G963, A964, C1065 or C1066 of mouse 18S rRNA (SEQ ID NO:23); a complementary sequence thereto; or a sequence that is at least 95% identical to the foregoing. ~~In one aspect, provided are~~Also disclosed are synthetic or isolated polynucleotides encoding a mammalian 18S rRNA that is resistant to pactamycin. In these polynucleotides, the pactamycin-resistance is conferred by one or more single residue substitutions in the 18S rRNA sequence. Also provided are fragments of such polynucleotides harboring the substitutions, complementary sequences, and substantially identical sequences. In some embodiments, the single residue substitution in the polynucleotide conferring pactamycin-resistance is at a position corresponding to G963, A964, C1065 or C1066 of mouse 18S rRNA (SEQ ID NO:23). In some preferred embodiments, the single residue substitution is at a position corresponding to position G963 of SEQ ID NO:23. Some of these polynucleotides contain a single residue substitution that corresponds to a G963A substitution in SEQ ID NO:23.

[0008] In some embodiments, the polynucleotide encodes a pactamycin resistant human 18S rRNA or mouse 18S rRNA. In some embodiments, the polynucleotide includes SEQ ID NO:23 except for a G963A substitution. In some related aspects, provided are mammalian 18S rRNA molecules encoded by the synthetic or isolated polynucleotides. In another aspect, provided are vectors harboring the polynucleotides that encode a pactamycin resistant 18S rRNA. In some of these vectors, the polynucleotide can further include the 5' ETS and ITS1 of the rDNA sequence. In some embodiments, the vectors further include a pol-I promoter or a cytomegalovirus (CMV) promoter. In some embodiments, the vectors further include the 3' ETS or an SV40 poly(A) signal. In another related aspect, host cells that harbor an expression vector are disclosed herein.

[0009] In another aspect, there is provided a method for identifying a mutation in 18S rRNA that alters ribosomal functions, comprising: (a) introducing an additional mutation to the synthetic polynucleotide of claim 1; (b) expressing the synthetic polynucleotide bearing the additional mutation in a host cell in the presence of pactamycin; and (c) detecting an alteration in the ribosomes of the host cell relative to that of a control cell expressing the synthetic polynucleotide without the additional mutation; thereby identifying the additional mutation as one altering ribosomal functions. ~~In another aspect, provided are~~Also disclosed are methods for identifying a mutation in 18S rRNA that alters ribosomal functions. The methods entail (a) introducing an additional mutation to a synthetic polynucleotide encoding a mammalian 18S rRNA that is resistant to pactamycin, wherein the pactamycin-resistance is conferred by one or more single residue substitutions in the 18S rRNA sequence, (b) expressing the synthetic polynucleotide bearing the additional mutation in a host cell in the presence of pactamycin, and (c) detecting an alteration in the ribosomes of the host cell relative to that of a control cell expressing the synthetic

polynucleotide without the additional mutation. These steps allow identification of the additional mutation as one altering ribosomal functions. Typically, the synthetic polynucleotide is present in an expression vector introduced into the host cell. In some embodiments, the pactamycin-resistant 18S rRNA harbors a single residue substitution at a position corresponding to G963, A964, C1065 or C1066 of mouse 18S rRNA (SEQ ID NO:23). For example, the 18S rRNA can harbor a substitution corresponding to a G963A substitution in SEQ ID NO:23. Some methods employ a synthetic polynucleotide comprising SEQ ID NO:23 except for a G963A substitution.

[0010] In another aspect, there is provided a method for producing ribosomes with enhanced translation efficiency in a mammalian cell, comprising: (a) introducing an additional mutation to the synthetic polynucleotide of claim 1; (b) introducing the synthetic polynucleotide bearing said additional mutation into a host mammalian cell; (c) culturing the cell in the presence of pactamycin; and (d) detecting enhanced translation efficiency in the cell relative to that of a control cell expressing the synthetic polynucleotide without the additional mutation; thereby producing ribosomes with enhanced translation efficiency. ~~In another aspect, provided~~ Also disclosed are methods for producing ribosomes with enhanced translation efficiency in a mammalian cell. These methods entail (a) introducing an additional mutation to a synthetic polynucleotide encoding a pactamycin-resistance mammalian 18S rRNA, wherein the pactamycin-resistance is conferred by one or more single residue substitutions in the 18S rRNA sequence, (b) introducing the synthetic polynucleotide bearing said additional mutation into a host mammalian cell, (c) culturing the cell in the presence of pactamycin, and (d) detecting enhanced translation efficiency in the cell relative to that of a control cell expressing the synthetic polynucleotide without the additional mutation. With these methods, ribosomes with enhanced translation efficiency can be obtained. Typically, the synthetic polynucleotide employed in these methods is present in an expression vector introduced into the host cell. In some embodiments, the pactamycin-resistant 18S rRNA harbors a single residue substitution at position corresponding to G963, A964, C1065 or C1066 of mouse 18S rRNA (SEQ ID NO:23). Some of the methods employ a 18s rRNA-encoding synthetic polynucleotide with a single residue substitution that corresponds to a G963A substitution in SEQ ID NO:23. For example, the methods can utilize a synthetic polynucleotide that includes SEQ ID NO:23 except for a G963A substitution. In various embodiments, translation efficiency can be determined by measuring the level of a specific polypeptide in the host cell.

[0011] In another aspect, provided is a kit comprising an expression vector comprising a synthetic polynucleotide encoding a mammalian 18S rRNA that is resistant to pactamycin, wherein the pactamycin-resistance is conferred by one or more single residue substitutions in the 18S rRNA sequence, as described herein. In one embodiment, the kit further includes a cell containing the expression vector.

[0012] In another aspect, there is provided a method for preferentially translating a recombinant mRNA, the method comprising: expressing the synthetic polynucleotide of claim 1 in a mammalian cell, wherein the synthetic polynucleotide has been altered to introduce one or more mutations that confer preferential binding of the 18S rRNA to the recombinant mRNA; providing the recombinant mRNA to the cell; and exposing the cell to pactamycin in an amount sufficient to reduce or eliminate protein synthesis from the cell's endogenous 40S ribosomal subunits, thereby largely restricting protein synthesis in the cell to 40S ribosomal subunits comprising the 18S rRNA expressed by the synthetic polynucleotide of claim 1 and preferentially translating the recombinant mRNA. ~~In another aspect, provided~~Also disclosed is a method for preferentially translating a recombinant mRNA, the method comprising expressing in a mammalian cell a polynucleotide encoding a mammalian 18S rRNA that is resistant to pactamycin, wherein the

WHAT IS CLAIMED IS:

1. A synthetic or isolated polynucleotide encoding a mammalian 18S rRNA that is resistant to pactamycin, wherein the pactamycin-resistance is conferred by one or more single residue substitutions in the 18S rRNA sequence; a fragment thereof harboring said substitutions; a complementary sequence thereto; or a substantially identical sequence of the foregoing.

2. The polynucleotide of claim 1, wherein the single residue substitution is at a position corresponding to G963, A964, C1065 or C1066 of mouse 18S rRNA (SEQ ID NO:23).

3. The polynucleotide of claim 1, wherein the single residue substitution is at a position corresponding to position G963 of SEQ ID NO:23.

4. The polynucleotide of claim 1, wherein the single residue substitution corresponds to a G963A substitution in SEQ ID NO:23.

5. The polynucleotide of claim 1, wherein the polynucleotide encodes human 18S rRNA or mouse 18S rRNA.

6. The polynucleotide of claim 1, wherein the polynucleotide comprises SEQ ID NO:23 except for a G963A substitution.

7. A mammalian 18S rRNA encoded by the polynucleotide of claim 1.

8. An expression vector expressing the polynucleotide of claim 1.

9. The vector of claim 8, wherein the polynucleotide further comprises the 5' ETS and ITS1 of the rDNA sequence.

10. The vector of claim 8, further comprises a pol-I promoter or a cytomegalovirus (CMV) promoter.

11. The vector of claim 8, further comprises the 3' ETS or an SV40 poly(A) signal.

12. A host cell harboring the expression vector of claim 8.

13. A kit comprising the expression vector of any one of claims 8-11 and/or a cell containing the expression vector.

14. A method for preferentially translating a recombinant mRNA, the method comprising:

expressing the synthetic polynucleotide of claim 1 in a mammalian cell, wherein the synthetic polynucleotide has been altered to introduce one or more mutations that confer preferential binding of the 18S rRNA to the recombinant mRNA;

providing the recombinant mRNA to the cell; and

exposing the cell to pactamycin in an amount sufficient to reduce or eliminate protein synthesis from the cell's endogenous 40S ribosomal subunits, thereby largely restricting protein synthesis in the cell to 40S ribosomal subunits comprising the 18S rRNA expressed by the synthetic polynucleotide of claim 1 and preferentially translating the recombinant mRNA.

15. A method for identifying a mutation in 18S rRNA that alters ribosomal functions, comprising:

(a) introducing an additional mutation to a synthetic polynucleotide encoding a mammalian 18S rRNA that is resistant to pactamycin, wherein the pactamycin-resistance is conferred by one or more single residue substitutions in the 18S rRNA sequence;

(b) expressing the synthetic polynucleotide bearing the additional mutation in a host cell in the presence of pactamycin; and

(c) detecting an alteration in the ribosomes of the host cell relative to that of a control cell expressing the synthetic polynucleotide without the additional mutation; thereby identifying the additional mutation as one altering ribosomal functions.

16. The method of claim 15, wherein the synthetic polynucleotide is present in an expression vector introduced into the host cell.

17. The method of claim 15, wherein the pactamycin-resistant 18S rRNA harbors a single residue substitution at a position corresponding to G963, A964, C1065 or C1066 of mouse 18S rRNA (SEQ ID NO:23).

18. The method of claim 15, wherein the single residue substitution corresponds to a G963A substitution in SEQ ID NO:23.

19. The method of claim 15, wherein the synthetic polynucleotide comprises SEQ ID NO:23 except for a G963A substitution.

20. A method for producing ribosomes with enhanced translation efficiency in a mammalian cell, comprising:

(a) introducing an additional mutation to a synthetic polynucleotide encoding a pactamycin-resistance mammalian 18S rRNA, wherein the pactamycin-resistance is conferred by one or more single residue substitutions in the 18S rRNA sequence;

(b) introducing the synthetic polynucleotide bearing said additional mutation into a host mammalian cell;

(c) culturing the cell in the presence of pactamycin; and

(d) detecting enhanced translation efficiency in the cell relative to that of a control cell expressing the synthetic polynucleotide without the additional mutation; thereby producing ribosomes with enhanced translation efficiency.

21. The method of claim 20, wherein the synthetic polynucleotide is present in an expression vector introduced into the host cell.

22. The method of claim 20, wherein the pactamycin-resistant 18S rRNA harbors a single residue substitution at position corresponding to G963, A964, C1065 or C1066 of mouse 18S rRNA (SEQ ID NO:23).

23. The method of claim 20, wherein the single residue substitution corresponds to a G963A substitution in SEQ ID NO:23.

24. The method of claim 20, wherein the synthetic polynucleotide comprises SEQ ID NO:23 except for a G963A substitution.

25. The method of claim 20, wherein translation efficiency is determined by measuring the level of a specific polypeptide in the host cell.

WHAT IS CLAIMED IS:

1. A synthetic or isolated polynucleotide encoding a mammalian 18S rRNA that is resistant to pactamycin, wherein the pactamycin-resistance is conferred by one or more single residue substitutions in the 18S rRNA sequence, wherein the single residue substitution is at a position corresponding to G963, A964, C1065 or C1066 of mouse 18S rRNA (SEQ ID NO:23); a complementary sequence thereto; or a sequence that is at least 95% identical to the foregoing.

2. The polynucleotide of claim 1, wherein the single residue substitution is at a position corresponding to position G963 of SEQ ID NO:23.

3. The polynucleotide of claim 1, wherein the single residue substitution corresponds to a G963A substitution in SEQ ID NO:23.

4. The polynucleotide of claim 1, wherein the polynucleotide encodes human 18S rRNA or mouse 18S rRNA.

5. The polynucleotide of claim 1, wherein the polynucleotide comprises SEQ ID NO:23 except for a G963A substitution.

6. A mammalian 18S rRNA encoded by the polynucleotide of claim 1.

7. An expression vector expressing the polynucleotide of claim 1.

8. The vector of claim 7, wherein the polynucleotide further comprises the 5' ETS and ITS1 of the rDNA sequence.

9. The vector of claim 7, further comprises a pol-I promoter or a cytomegalovirus (CMV) promoter.

10. The vector of claim 7, further comprises the 3' ETS or an SV40 poly(A) signal.

11. A host cell harboring the expression vector of claim 7.

12. A kit comprising the expression vector of any one of claims 7-10 and/or a cell containing the expression vector.

13. A method for preferentially translating a recombinant mRNA, the method comprising:

expressing the synthetic polynucleotide of claim 1 in a mammalian cell, wherein the synthetic polynucleotide has been altered to introduce one or more mutations that confer preferential binding of the 18S rRNA to the recombinant mRNA;

providing the recombinant mRNA to the cell; and

exposing the cell to pactamycin in an amount sufficient to reduce or eliminate protein synthesis from the cell's endogenous 40S ribosomal subunits, thereby largely restricting protein synthesis in the cell to 40S ribosomal subunits comprising the 18S rRNA expressed by the synthetic polynucleotide of claim 1 and preferentially translating the recombinant mRNA.

14. A method for identifying a mutation in 18S rRNA that alters ribosomal functions, comprising:

(a) introducing an additional mutation to the synthetic polynucleotide of claim 1

(b) expressing the synthetic polynucleotide bearing the additional mutation in a host cell in the presence of pactamycin; and

(c) detecting an alteration in the ribosomes of the host cell relative to that of a control cell expressing the synthetic polynucleotide without the additional mutation; thereby identifying the additional mutation as one altering ribosomal functions.

15. The method of claim 14, wherein the synthetic polynucleotide is present in an expression vector introduced into the host cell.

16. The method of claim 14, wherein the single residue substitution corresponds to a G963A substitution in SEQ ID NO:23.

17. The method of claim 14, wherein the synthetic polynucleotide comprises SEQ ID NO:23 except for a G963A substitution.

18. A method for producing ribosomes with enhanced translation efficiency in a mammalian cell, comprising:

(a) introducing an additional mutation to the synthetic polynucleotide of claim 1;

(b) introducing the synthetic polynucleotide bearing said additional mutation into a host mammalian cell;

(c) culturing the cell in the presence of pactamycin; and

(d) detecting enhanced translation efficiency in the cell relative to that of a control cell expressing the synthetic polynucleotide without the additional mutation; thereby producing ribosomes with enhanced translation efficiency.

19. The method of claim 18, wherein the synthetic polynucleotide is present in an expression vector introduced into the host cell.

20. The method of claim 18, wherein the single residue substitution corresponds to a G963A substitution in SEQ ID NO:23.

21. The method of claim 18, wherein the synthetic polynucleotide comprises SEQ ID NO:23 except for a G963A substitution.

22. The method of claim 18, wherein translation efficiency is determined by measuring the level of a specific polypeptide in the host cell.