CLAIMS

We claim:

1. A circular RNA precursor, comprising from 5' to 3' direction: the 3’ self-splicing intron fragment; a nucleotide sequence of interest; and the 5’ self-splicing intron fragment,

wherein the 3’ self-splicing intron fragment and the 5’ self-splicing intron fragment in combination retain the self-splicing activity of a self-splicing intron and allow generation of a circular RNA which is formed by the nucleotide sequence of interest through the self-splicing of the circular RNA precursor and which comprises at least one protein-coding sequence;

wherein the nucleotide sequence of interest comprises from 5' to 3' direction: a protein-coding sequence fragment II, a translation initiation element such as an internal ribosome entry site (IRES), and a protein-coding sequence fragment I;

wherein the protein-coding sequence fragment I is the 5’ terminal portion of the at least one protein-coding sequence, and the protein-coding sequence fragment II is the 3’ terminal portion of the at least one protein-coding sequence, and

the protein-coding sequence fragment I comprises a ribozyme recognition site I located at its 3’ end, the protein-coding sequence fragment II comprises a ribozyme recognition site II located at its 5’ end, wherein in the generated circular RNA the ribozyme recognition site I is connected with the ribozyme recognition site II, and thus the protein-coding sequence fragment I is connected with the protein-coding sequence fragment II to form the at least one protein-coding sequence.

2. The circular RNA precursor of claim 1, wherein the self-splicing intron is cyanobacterium Anabaena Group I Intron such as AnaX having a nucleotide sequence as set forth in SEQ ID NO. 1, and the intron internal split site II and the intron internal split site I respectively correspond to nucleotide A at position 115 and nucleotide A at position 116 from 5’ end of SEQ ID NO. 1.

3. The circular RNA precursor of claim 1, wherein the self-splicing intron is Staphylococcus phage Twort Group I Intron (Twort) which has a nucleotide sequence as set forth in SEQ ID NO. 3, and

1) the intron internal split site II and the intron internal split site I respectively correspond to nucleotide U at position 70 and nucleotide A at position 71 from the 5′ end of SEQ ID NO. 3; or

2) the intron internal split site II and the intron internal split site I respectively correspond to nucleotide U at position 103 and nucleotide A at position 104 from the 5′ end of SEQ ID NO. 3; or

3) the intron internal split site II and the intron internal split site I respectively correspond to nucleotide A at position 132 and nucleotide U at position 133 from the 5′ end of SEQ ID NO. 3;

or

4) the intron internal split site II and the intron internal split site I respectively correspond to nucleotide U at position 173 and nucleotide C at position 174 from the 5' end of SEQ ID NO. 3; or

5) the intron internal split site II and the intron internal split site I respectively correspond to nucleotide A at position 206 and nucleotide A at position 207 from the 5' end of SEQ ID NO. 3; or

6) the intron internal split site II and the intron internal split site I respectively correspond to nucleotide G at position 226 and nucleotide U at position 227 from the 5' end of SEQ ID NO. 3; or

7) the intron internal split site II and the intron internal split site I respectively correspond to nucleotide U at position 23 and nucleotide A at position 24 from the 5' end of SEQ ID NO. 3; or

8) the intron internal split site II and the intron internal split site I respectively correspond to nucleotide A at position 82 and nucleotide A at position 83 from the 5' end of SEQ ID NO. 3.

4. The circular RNA precursor of any one of claims 1-3, wherein the generated circular RNA comprises at least two protein-coding sequences, and a further translation initiation element, 2A sequence or spacer may be linked between any two adjacent protein-coding sequences, and the translation initiation element comprises: IRES sequence, 5' UTR sequence, Kozak sequence, a sequence containing m6A, a sequence complementary to ribosome 18S rRNA, or any combination thereof.

5. The circular RNA precursor of any one of claims 1-4, wherein the ribozyme recognition site I and the ribozyme recognition site II are configured to be capable of forming a stem-loop structure, e.g., upon self-splicing for circularization, wherein the loop of the stem-loop structure comprises the splicing junction.

6. The circular RNA precursor of any one of claims 1-5, wherein the IRES comprises a nucleotide sequence set forth in one of SEQ ID NOs: 120-526, or comprises a nucleotide sequence having at least 75%, e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% , or at least 99% sequence identity to one of SEQ ID NOs: 120-526.

7. The circular RNA precursor of any one of claims 1-6, wherein the at least one protein-coding sequence encodes a protein of eukaryotic, prokaryotic or viral origin, for example, a protein

for therapeutic or diagnostic use, in particular a protein of mammalian or human origin.

8. The circular RNA precursor of any one of claims 1-7, wherein the at least one protein-coding sequence encodes an aquaporin.

9. The circular RNA precursor of claim 8, wherein the aquaporin is AQP-1 protein or a protein having the same activity as the AQP-1 protein and derived from the AQP-1 protein through substitution, deletion or addition of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids in the amino acid sequence of the AQP-1 protein.

10. The circular RNA precursor of any one of claims 1-9, which comprises a nucleotide sequence selected from any one of SEQ ID NO: 530, 531 and 533-534, or a nucleotide sequence having at least 50%, at least 60%, at least 70%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% , or at least 99% sequence identity to any one of SEQ ID NO: 530, 531 and 533-534.

11. A nucleic acid vector for generating a circular RNA molecule, said vector comprises a coding sequence of the circular RNA precursor of any one of claims 1-10.

12. The nucleic acid vector of claim 11, which further comprises an RNA polymerase promoter sequence operably linked to the coding sequence of the circular RNA precursor.

13. A circular RNA, which is prepared from the circular RNA precursor of any one of claims 1-10, preferably said circular RNA has a duration of in vivo expression of at least 24 hours, for example, 36 hours, 48 hours, 72 hours, 96 hours, 5 days, 10 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days or more.

14. The circular RNA of claim 13, which comprises or has a nucleotide sequence of any one of SEQ ID NO: 543-558, or a nucleotide sequence having at least 50%, at least 60%, at least 70%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NO: 543-558.

15. A pharmaceutical composition comprising circular RNA of claim 13 or 14, and a pharmaceutically acceptable carrier.

16. Use of the circular RNA of claim 13 or 14 or the pharmaceutical composition of claim 15 in the manufacture of a medicament for preventing or treating a disease or condition in a subject.

17. The use of claim 16, wherein the disease or condition is xerostomia, xerophthalmia, or Sjogren's syndrome.