

The authors have compiled an informative and insightful review of the mechanism and detection of alternative splicing events in circular RNAs. This reviewer found the topic timely and of great interest, and the article informative. Overall, I recommend this manuscript for publication pending minor edits. A few suggestions listed below would help to provide a bit more in depth detail and answer questions that naturally arise from the article's discussion. Also, moderate English editing would be helpful to clarify the point of several sentences.

- Discuss factors that could cause an up-regulation of circRNA that would cause an underproduction of linear RNA, and the role alternative splicing might be playing.
- Discuss adverse effects that lower concentrations of linear RNA might cause and the role that alternative splicing could mediate in pathogenesis, since circRNAs are related to a wide variety of diseases and the authors identify alternative splicing as central factor to the bioavailability of circRNAs.
- line 37-38: "Only partial of circRNAs show evolutionary conservation, most of them have tissue- or developmental stage specificity". The developmental stage or tissue specificity does not necessarily indicate that they are not evolutionarily conserved.
- Line 106: "Experimental methods, such as northern blotting, are able to validate this phenomenon". It is true that northern blot can be used to validate circRNAs, but it is an obsolete technique, currently validations of circRNAs are done by reverse transcription-PCR (RT-qPCR) or RNA-sequencing (RNA-Seq), among others. I recommend to the authors include "William R. Jeck and Norman E. Sharpless, Nat Biotechnol, 2015" and "Beltran-Garcia et al, Cells, 2020"
- Line 125-126: "ongoing investigations have begun to focus on factors which are linked to AS." Include some examples to validate the statement.

- Review the manuscript and cite the abbreviations the first time they appear in the text, for example: line 104: "alternative back-splice sites ..." line 106: "alternative back-splice sites (ABS) ..."
- How quickly are the circRNA concentrations adjusted, and could the concentration adjustments affect the validation of the circRNAs by bioinformatic or experimental methods?
- Can linear RNA become circularized?