

## **Deconstructing internal ribosome entry site elements: an update of structural motifs and functional divergences**

Gloria Lozano, Rosario Francisco-Velilla and Encarnacion Martinez-Salas

### **Article citation details**

*Open Biol.* **8**: 180155.

<http://dx.doi.org/10.1098/rsob.180155>

### **Review timeline**

Original submission: 30 August 2018  
1st revised submission: 3 October 2018  
2nd revised submission: 30 October 2018  
Final acceptance: 30 October 2018

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

## Review History

RSOB-18-0155.R0 (Original submission)

Review form: Reviewer 1

### **Recommendation**

Accept with minor revision (please list in comments)

Are each of the following suitable for general readers?

- a) **Title**  
Yes
  
- b) **Summary**  
Yes
  
- c) **Introduction**  
Yes

**Is the length of the paper justified?**

Yes

**Should the paper be seen by a specialist statistical reviewer?**

No

**Is it clear how to make all supporting data available?**

Not Applicable

**Is the supplementary material necessary; and if so is it adequate and clear?**

Not Applicable

**Do you have any ethical concerns with this paper?**

No

**Comments to the Author**

Review RSOB-18-0155

Gloria Lozano, Rosario Francisco-Velilla and Encarna Martinez-Salas:

"Deconstructing IRES elements: an update of structural motifs and functional divergences"

In this review, the authors provide a very interesting and comprehensive overview over viral and cellular internal ribosome entry site (IRES) elements, with a focus on the possible modular organization of IRES elements by using "building blocks" of small RNA segments that act with primary and/or secondary structure elements. This is a very interesting view and, in my opinion, warrants publication in Open Biology.

The text develops several aspects of IRES elements over long passages, while, in relation that, the actual section on "building blocks" is a bit short. However, this largely reflects the current status of research.

I have only a few suggestions:

1. In Fig. 1B, the authors could take the chance to mark some of the building blocks they mention in the text.
2. In the Type II IRES in Fig. 1B, the domains may be labeled also with numbers (2, 3 etc., which actually are mentioned in the text).
3. I was surprised to see PCBP2 acting on Type II IRES domain 3. Perhaps I missed that, but the literature cited for that (Andreev, Kafasla, Sweeney) does not support that statement. Perhaps PCBP2 interaction refers to Type I IRES elements? The authors may either better support that statement or remove the PCBP2 label from Fig. 1B.
4. In Fig. 2, the highlighting colours may be somehow changed for enhanced visibility.
5. "Evidence" has no plural form. Please correct "evidences" (repeatedly in the text) to "evidence".
6. In line 143, "polypyrimidin-binding protein (PTB) may be corrected to "polypyrimidine-tract binding protein" (an additional "e" and a "tract").

7. The statement in line 157 that SL I of HCV is involved in replication is incomplete, also SL II is involved in replication regulation (overlapping with its function in translation regulation).

8. When it comes to controls for checking if an RNA segment indeed is an IRES element, the authors should not miss to cite the useful experimental suggestions by Richard Lloyd's group (van Eden et al., 2004) and perhaps also comment on the use of these criteria in recent high throughput experiments.

## Decision letter (RSOB-18-0155.R0)

25-Sep-2018

Dear Dr Martinez-Salas

We are pleased to inform you that your manuscript RSOB-18-0155 entitled "Deconstructing IRES elements: an update of structural motifs and functional divergences" has been accepted by the Editor for publication in Open Biology. The reviewer(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, we invite you to respond to the reviewer's comments and revise your manuscript.

Please submit the revised version of your manuscript within 14 days. If you do not think you will be able to meet this date please let us know immediately and we can extend this deadline for you.

To revise your manuscript, log into <https://mc.manuscriptcentral.com/rsob> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, please revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referee(s).

Please see our detailed instructions for revision requirements  
<https://royalsociety.org/journals/authors/author-guidelines/>.

Before uploading your revised files please make sure that you have:

- 1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".
- 2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and meet our ESM criteria (see <http://royalsocietypublishing.org/instructions-authors#question5>). All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rsob.2016[last 4 digits of e.g. 10.1098/rsob.20160049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript. Please try to write in simple English, avoid jargon, explain the importance of the topic, outline the main implications and describe why this topic is newsworthy.

#### Images

We require suitable relevant images to appear alongside published articles. Do you have an image we could use? Images should have a resolution of at least 300 dpi, if possible.

#### Data-Sharing

It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see <http://royalsocietypublishing.org/site/authors/policy.xhtml#question6> for more details.

#### Data accessibility section

To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:

- DNA sequences: Genbank accessions F234391-F234402
- Phylogenetic data: TreeBASE accession number S9123
- Final DNA sequence assembly uploaded as online supplemental material
- Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311

Once again, thank you for submitting your manuscript to Open Biology, we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,

The Open Biology Team  
<mailto:openbiology@royalsociety.org>

Reviewer's Comments to Author:

Referee: 1

Comments to the Author(s)  
Review RSOB-18-0155

Gloria Lozano, Rosario Francisco-Velilla and Encarna Martinez-Salas:  
"Deconstructing IRES elements: an update of structural motifs and functional divergences"

In this review, the authors provide a very interesting and comprehensive overview over viral and cellular internal ribosome entry site (IRES) elements, with a focus on the possible modular organization of IRES elements by using "building blocks" of small RNA segments that act with primary and/or secondary structure elements. This is a very interesting view and, in my opinion, warrants publication in Open Biology.

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8. When it comes to controls for checking if an RNA segment indeed is an IRES element, the authors should not miss to cite the useful experimental suggestions by Richard Lloyd's group (van Eden et al., 2004) and perhaps also comment on the use of these criteria in recent high throughput experiments.

## Author's Response to Decision Letter for (RSOB-18-0155.R0)

See Appendix A.

# RSOB-18-0155.R1

## Review form: Reviewer 1

### Recommendation

Accept as is

### Are each of the following suitable for general readers?

- a) **Title**  
Yes
- b) **Summary**  
Yes
- c) **Introduction**  
Yes

### Is the length of the paper justified?

Yes

### Should the paper be seen by a specialist statistical reviewer?

No

### Is it clear how to make all supporting data available?

Yes

### Is the supplementary material necessary; and if so is it adequate and clear?

Yes

### Do you have any ethical concerns with this paper?

No

### Comments to the Author

The authors have followed my recommendations, I am fine with the changes.

## Decision letter (RSOB-18-0155.R1)

30-Oct-2018

Dear Dr Martinez-Salas

We are pleased to inform you that your manuscript RSOB-18-0155.R1 entitled "Deconstructing IRES elements: an update of structural motifs and functional divergences" has been accepted by the Editor for publication in Open Biology.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it within the next 10 working days. Please let us know if you are likely to be away from e-mail contact during this time.

Thank you for your fine contribution. On behalf of the Editors of Open Biology, we look forward to your continued contributions to the journal.

Sincerely,

The Open Biology Team  
mailto: [openbiology@royalsociety.org](mailto:openbiology@royalsociety.org).

## Decision letter (RSOB-18-0155.R2)

30-Oct-2018

Dear Dr Martinez-Salas

We are pleased to inform you that your manuscript entitled "Deconstructing IRES elements: an update of structural motifs and functional divergences" has been accepted by the Editor for publication in Open Biology.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it within the next 10 working days. Please let us know if you are likely to be away from e-mail contact during this time.

Thank you for your fine contribution. On behalf of the Editors of Open Biology, we look forward to your continued contributions to the journal.

Sincerely,

The Open Biology Team  
mailto: [openbiology@royalsociety.org](mailto:openbiology@royalsociety.org)



CENTRO DE BIOLOGIA MOLECULAR  
"SEVERO OCHOA"

## Appendix A

Royal Society's Open Biology  
Editorial office

October 3th, 2018

Dear Dr. Glover,

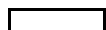
We have submitted a revised version of the review entitled "Deconstructing IRES elements: an update of structural motifs and functional divergences" by Gloria Lozano, Rosario Francisco-Velilla, and myself, to be considered for publication in Open Biology.

We were very happy to see the positive comments of yourself and the reviewer. To address his/her points, we have modified the manuscript to include revised versions of Figures 1 and 2. A modification to the text was included in page 9 to better explain the section on RNA building blocks. All minor points were corrected. As a consequence of the changes inserted in the text several references were added.

A point-by-point response is shown in the next pages.

We thank you for your attention and look forward to hearing from you,

Encarna Martínez-Salas  
Centro de Biología Molecular Severo Ochoa  
email: [emartinez@cbm.csic.es](mailto:emartinez@cbm.csic.es)  
<http://web4.cbm.uam.es/joomla-rl/index.php/en/scientific-departments/genome-dynamics-and-function?id=%20536>



E-mail: [emartinez@cbm.csic.es](mailto:emartinez@cbm.csic.es)

**Centro de Biología Molecular "Severo Ochoa"**  
Consejo Superior de Investigaciones Científicas  
Universidad Autónoma de Madrid  
Cantoblanco, 28049. MADRID  
TEL.: 91 1964619  
FAX: 91 1964420





RESPONSE:

*In this review, the authors provide a very interesting and comprehensive overview over viral and cellular internal ribosome entry site (IRES) elements, with a focus on the possible modular organization of IRES elements by using "building blocks" of small RNA segments that act with primary and/or secondary structure elements. This is a very interesting view and, in my opinion, warrants publication in Open Biology.*

*The text develops several aspects of IRES elements over long passages, while, in relation that, the actual section on "building blocks" is a bit short. However, this largely reflects the current status of research.*

- Thanks for this comment. We have expanded this section including references to two examples of artificial IRES elements (see page 9)

*I have only a few suggestions:*

1. *In Fig. 1B, the authors could take the chance to mark some of the building blocks they mention in the text.*

-Done as requested.

2. *In the Type II IRES in Fig. 1B, the domains may be labeled also with numbers (2, 3 etc., which actually are mentioned in the text).*

-Done as requested.

3. *I was surprised to see PCBP2 acting on Type II IRES domain 3. Perhaps I missed that, but the literature cited for that (Andreev, Kafasla, Sweeney) does not support that statement. Perhaps PCBP2 interaction refers to Type I IRES elements? The authors may either better support that statement or remove the PCBP2 label from Fig. 1B.*

- Thanks for this comment. The figure was correct. However, as indicated in the text PCBP2 binds to both type I and type II although we failed to cite references for type II, which are now included.

4. *In Fig. 2, the highlighting colours may be somehow changed for enhanced visibility.*

-Done as requested.

5. *"Evidence" has no plural form. Please correct "evidences" (repeatedly in the text) to "evidence".*

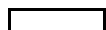
-Done as requested.

6. *In line 143, "polypyrimidin-binding protein (PTB) may be corrected to "polypyrimidine-tract binding protein" (an additional "e" and a "tract").*

-Done as requested.

7. *The statement in line 157 that SL I of HCV is involved in replication is incomplete, also SL II is involved in replication regulation (overlapping with its function in translation regulation).*

- Thanks for this comment. We have modified the sentence, and the corresponding references are included.

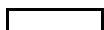




CENTRO DE BIOLOGIA MOLECULAR  
"SEVERO OCHOA"

8. *When it comes to controls for checking if an RNA segment indeed is an IRES element, the authors should not miss to cite the useful experimental suggestions by Richard Lloyd's group (van Eden et al., 2004) and perhaps also comment on the use of these criteria in recent high throughput experiments.*

- We fully agree with the reviewer. The reference by R. Lloyd team is now included.



E-mail: [emartinez@cbm.csic.es](mailto:emartinez@cbm.csic.es)

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