Journal of Cell Science

SART3 associates with a post-splicing complex

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DOI: 10.1242/jcs.260380

Editor: Maria Carmo-Fonseca

Review timeline
Original submission: 27 June 2022
Editorial decision: 1 August 2022
First revision received: 8 December 2022
Accepted: 10 December 2022

Original submission

First decision letter

MS ID#: JOCES/2022/260380

MS TITLE: SART3 associates with a post-splicing complex

AUTHORS: Klara Klimesova, Cyril Barinka, and David Stanek
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

In the present study, Klimesova et al report a very thorough and detailed description of SART3 involvement in splicesomal complexes, including a dissection of SART3 protein domains. Both by immunoprecipitation and gradient centrifugation assays, they reveal the importance of N-terminal domain to circunscribe SART3 to a specific U2 snRNP at postsplicing and enable U6 snRNA recycling.

Taking into account the progressive advances made to the field of spliceosome asssembly in terms of composition, structure and function over the last decade, the present study contributes to understand the recycling process that allows the rearrangement of such a complex machinery for a subsequent round of splicing.

Comments for the author

In Fig S1, is it possible to quantify size or number of GFP foci with the different mutants? Or even residence time in Cajal bodies with FRAP? Also, C-SART3-GFP seems to have a nucleolar localization, any reason why? In several blots, SART3-GFP and N-SART3 show 2 bands. Can the authors speculate about this?
Is it possible that U2 snRNP interaction with N-SART3 is higher only because N-SART3 expression and IP are higher? Particularly when comparing with the wt full length, protein? Could this variant be more stable for a particular biological reason?

Overall, it would strengthen the results to monitor splicing efficiency in the presence of the different mutants(either in an in vitro assay or endogenous events in cultured cells).

In Fig 3, blots for PRPF8, PRPF19 and DHX15 yied very low signal. Is it possible for the authors to show the blots from the first IP? Mass spec would be very informative in this respect although likely beyond the scope of the present manuscript.

In Fig 4, authors might want to discuss the results of siTFIP11 taking into account the report from Ducheim et al from November 2021.

Minor points
In Fig 1, it would make it clearer to indicate that the first construct is wt/full length. Similarly, in fig 2E, to specify next to protein name the snRNP they belong to.
Some phrases in the introduction would benefit from re-writing, for example:
avoid terms like "has to be reannealed", "needs to be reassembled".

Reviewer 2

Advance summary and potential significance to field

In this manuscript, Klimesova et al investigate the potential function(s) of SART3 in the splicing complex. The advance made in this article is that SART3 associates with a postsplicing complex to presumably regulate U6 snRNA recycling. Given that the molecular events regulating the disassembly and recycling of the postsplicing complex remain unclear, this new data shed new light on the function of SART3 in regulating this process.

Comments for the author

1) The data in Figure 1B suggest that the RRMs of SART3 are not involved in the association with U2 snRNP. To further investigate the SART3-U2 snRNP interaction, the authors could investigate whether U2 snRNP polypeptides and SART3 directly interact with each other by performing GST-pull-down experiments with U2-GST-tagged recombinant proteins. Similarly, the authors could test the interaction between SART3 and DHX15 using recombinant proteins or yeast two-hybrid assay. Also, have the authors experimentally verified the dependence of the U2 RNA on these interactions?

2) Fig. 3B. According to the results described in previous figures, I would expect to have more PRPF8 and DHX15 proteins immunoprecipitated using GFP-tagged N-SART3 than GFP-tagged SART3. This does not appear to be the case even with high recovery of N-SART3, especially as regards to the RNA helicase DHX15. Please clarify.

3) In my opinion, the results shown in Figure 4 are not as easily interpreted as the authors suggest. As the authors explain in the Introduction section of the manuscript, our knowledge of spliceosome disassembly and snRNP recycling derives primarily from work done in yeast with the NTR complex. TFIP11 and DHX15 have been implicated in snRNP recycling; however, recent data have shown

DHX15-independent functions for TFIP11. Work by Duchemin et al (Nat. Commun. 2021, 12:6648) has shown that TFIP11 regulates 2'-O-methylation of the U6 snRNA, U4/U6.U5 tri-snRNP formation and spliceosome assembly. Therefore, the authors should consider other possible explanations for their results and properly cite this article. In this line, the authors may consider testing whether DHX15 depletion affects the interaction of SART3 with the U2 snRNP complex.

Minor
4) The authors should show the position of SNRPB2 in the gradients of Fig. 2D and 3D by western blotting.

5) From Fig. S1 the localization of the SART3-GFP constructs is not entirely clear. Please provide higher-magnification insets of the features of interest.

First revision

Author response to reviewers' comments

RESPONSE TO REVIEWERS' COMMENTS

Reviewer #1

We thank the reviewer for positive comments on our work and for constructive suggestions that significantly improved our manuscript. Please, find below the point-by-point answer to your comments.

In Fig S1, is it possible to quantify size or number of GFP foci with the different mutants?

We quantified accumulation of individual SART3-GFP variants in Cajal bodies and these new data are now presented in Fig. S1B. The quantification confirms the microscopy images and shows that deletion of the N-terminal part of SART3 containing the HAT repeats or elimination of self-interacting HAT12 repeat (deltaCC-SART3-GFP construct) considerably reduces Cajal body localization. N-SART3-GFP Cajal body localization was also reduce. However, we provide evidence that coilin and likely also Cajal bodies are not important for SART3-U2 interaction (Fig. 2A) and we did not consider this effect particularly relevant for our study.

Or even residence time in Cajal bodies with FRAP?

We understand that these questions are interesting for a general SART3 function. However, in this manuscript we mainly focused on SART3-U2/post-splicing complex association. We showed that coilin does not mediate this interaction and thus this interaction most likely does not occur in Cajal bodies (Fig. 2A). We therefore did not focus on SART3 and Cajal bodies and did not perform FRAP experiments as suggested.

Also, C-SART3-GFP seems to have a nucleolar localization, any reason why?

The GFP-NLS shows very similar localization as C-SART3-GFP (Stanek et al. 2003). We therefore believe that this localization is unspecific and not biologically relevant. We added a sentence to the text to mention this point: "Consistent with previous observations, the SART3 construct lacking the HAT domain failed to accumulate in Cajal bodies and localized to nucleoplasm and nucleoli, which is likely unspecific because GFP-NLS exhibit similar localization (Stanek et al., 2003)." (p. 5, top paragraph). As we did not use this construct further in this study, we did not elaborate on this finding.

In several blots, SART3-GFP and N-SART3 show 2 bands. Can the authors speculate about this?

The reviewer is correct that we sometimes observe two bands. They likely represent products of a partial degradation of SART3-GFP proteins but we never investigated this phenomenon any deeper.

The smaller proteins represent only minor part of total SART3/N-SART3-GFP proteins expressed in cells and we therefore assume that they do not considerably affect the outcome of our experiments.

Is it possible that U2 snRNP interaction with N-SART3 is higher only because N-SART3 expression and IP are higher? Particularly when comparing with the wt, full length, protein? Could this variant be more stable for a particular, biological reason?

The reviewer is correct that N-SART3-GFP sometimes exhibits better expression than WT SART3-GFP, which can contribute to the observed changes in the interaction with U2/post-splicing complex. However, we detected better interaction of U2-specific proteins also in the case when the same or very similar amounts of N-SART3-GFP and WT SART3-GFP were immunoprecipitated (e.g. Figs. 3A, 4D). We therefore assume that the observed changes are mainly driven by enhanced association of N-SART3 with U2/post-splicing complexes. As far we know, there is no biological relevant reason why N-SART3-GFP would be more stable than the full-length protein. The N-SART3-construct is smaller, which might result in better transfection efficiency and thus better expression.

Overall, it would strengthen the results to monitor splicing efficiency in the presence of the different mutants(either in an in vitro assay or endogenous events in cultured cells).
We monitored efficiency of splicing of seven genes as suggested by the reviewer. Expression of neither of the SART3-GFP construct changed significantly splicing efficiency of these genes. These new data are now presented in Fig. S1D.

In Fig 3, blots for PRPF8, PRPF19 and DHX15 yied very low signal. Is it possible for the authors to show the blots from the first IP? Mass spec would be very informative in this respect although likely beyond the scope of the present manuscript.

We show the western blot after 1st immunoprecipitation with N-SART3/SART3-TEV-GFP in new Fig. 3A. It shows nice co-precipitation of U2 (SNRPA1) and U5-specific (PRPF8) proteins. However, signals from co-purified DHX15 and PRPF19 were rather low, which indicates that the association with these proteins is either weaker than with snRNP proteins or more transient. Based on following experiments (in vitro pull-downs and immunoprecipitation after RNase treatment (Fig. 4 A and B), we speculate that the second model is correct and SART3 interacts with DHX15 transiently. Thank you for the suggestion!

In Fig 4, authors might want to discuss the results of siTFIP11 taking into account the report from Ducheim et al from November 2021.
Thank you for pointing this out, we now discuss TFIP11 knockdown results more thoroughly in the first paragraph of Discussion (p. 9).

Minor points
In Fig 1, it would make it clearer to indicate that the first construct is wt/full length.

Done.

Similarly, in fig 2E, to specify next to protein name the snRNP they belong to.

Done.

Some phrases in the introduction would benefit from re-writing, for example: avoid terms like "has to be reannealed", "needs to be reassembled".

We revised the Introduction as suggested, thank you!

Reviewer #2

We thank the reviewer for positive comments on our work and for constructive suggestions that significantly improved our manuscript. Please, find below the point-by-point answer to your comments.

1) The data in Figure 1B suggest that the RRMs of SART3 are not involved in the association with U2 snRNP. To further investigate the SART3-U2 snRNP interaction, the authors could investigate whether U2 snRNP polypeptides and SART3 directly interact with each other by performing GST-pull-down experiments with U2-GST-tagged recombinant proteins. Similarly, the authors could test the interaction between SART3 and DHX15 using recombinant proteins or yeast two-hybrid assay.

We performed GST pull-down with recombinant N-SART3-GST as suggested by the reviewer. Expression of SNRPA1 was repeatedly extremely low and we could not assessed the interaction. We did not detect any considerable pull-down of SNRPB2. However, we revealed the interaction between N-SART3 and DHX15, either with the full-length protein as well as with the N-terminal part of DHX15 (aa1-320). These new data are shown in Fig. 4A.

Also, have the authors experimentally verified the dependence of the U2 RNA on these interactions?
We performed immunoprecipitation with SART3/N-SART3-GFP after an RNase treatment. While co-precipitation of both U2 snRNP-specific proteins (SNRPA1/B2) was completely abolished, interaction with DHX15 was only partially affected. These data are consistent with the GST pull-down experiment and indicate that DHX15 is an SART3 interaction partner in the post-splicing complex. The RNaseA experiment is presented in new Fig. 4B.

2) Fig. 3B. According to the results described in previous figures, I would expect to have more PRPF8 and DHX15 proteins immunoprecipitated using GFP-tagged N-SART3 than GFP-tagged SART3. This does not appear to be the case even with high recovery of N-SART3, especially as regards to the RNA helicase DHX15. Please clarify.

The reviewer is correct that based on previous results one could expect higher precipitation of DHX15 and PRPF8 with N-SART3-GFP than WT SART3-GFP in the double immunoprecipitation experiment. To get more insight into association of different proteins with SART3/N-SART3, we performed normal single immunoprecipitation with SART3/N-SART3-TEV-GFP (new Fig. 3A). Similarly to U2-specific proteins, PRPF8 co-purified stronger with N-SART3-GFP than with WT SART3-GFP. However, we did not observe considerable difference between co-precipitation of DHX15 between WT SART3-GFP and N-SART3-GFP. We propose, that under normal conditions WT SART3 interacts transiently with the post-splicing complex and then leaves with U6 snRNA. When the U6-interacting C-terminal domain is removed, N-SART3 is not able to leave with U6 and stays artificially associated with post-splicing U2 and/or U5 snRNPs, which contain only residual amount of DHX15. That would explain higher co-precipitation of U2 and U5 markers but not DHX15 with N-SART3-GFP. This model would be consistent also with the results of double IP (Fig. 3B) where we isolate complexes containing both SART3/N-SART3-GFP and U2 snRNP. We assume, that in the case of N-SART3, the majority of these complexes represents post-splicing U2 snRNP-N-SART3 particle, which contains rather limited amount of other splicing factors (PRPF8, DHX15). These factors are present together in a not-yest-disassembled post-splicing complex and both WT SART3 and N-SART3 interact with the post-splicing complex to as similar extend. We added this interpretation to Discussion (p. 9, bottom). However, I have to admit that our understanding of post-splicing complex disassembly and the fate of individual post-splicing snRNPs is currently limited.

3) In my opinion, the results shown in Figure 4 are not as easily interpreted as the authors suggest. As the authors explain in the Introduction section of the manuscript, our knowledge of spliceosome disassembly and snRNP recycling derives primarily from work done in yeast with the NTR complex. TFIP11 and DHX15 have been implicated in snRNP recycling; however, recent data have shown DHX15-independent functions for TFIP11. Work by Duchemin et al (Nat. Commun. 2021, 12:6648) has shown that TFIP11 regulates 2'-O-methylation of the U6 snRNA, U4/U6.U5 tri-snRNP formation and spliceosome assembly. Therefore, the authors should consider other possible explanations for their results and properly cite this article. In this line, the authors may consider testing whether DHX15 depletion affects the interaction of SART3 with the U2 snRNP complex.

Thank you for pointing this out. We now discuss TFIP11 knockdown results more thoroughly in the first paragraph of Discussion (p. 9, middle of the paragraph). We also performed DHX15 RNAi knockdown as suggested. We observed reduced association of U2-specific proteins with N-SART3-

GFP, which is consistent with our conclusion, that DHX15 is important for SART3 interaction with the post-splicing complex.

Minor
4) The authors should show the position of SNRPB2 in the gradients of Fig. 2D and 3D by western blotting.

Done. These results are now included in Figs. S2C and S3C.

5) From Fig. S1 the localization of the SART3-GFP constructs is not entirely clear. Please provide higher-magnification insets of the features of interest.

Done. We included magnified insets of selected Cajal bodies in Fig. S1A and quantified accumulation of individual SART3-GFP constructs in Cajal bodies (new Fig. S1B).

Second decision letter

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ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.