

Peer-Review Record:

CRISPR-Cas Adaptive Immune Systems of the Sulfolobales: Unravelling Their Complexity and Diversity

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Reviewer 1: Anonymous

Reviewer 2: Anonymous

Editor: Hans-Peter Klenk, Michael W. W. Adams and Helga Stan-Lotter

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First Round of Evaluation

Round 1: Reviewer 1 Report

This is a nice review on CRISPR activities in Sulfolobales, including also an overview of viruses and plasmids in the introduction. The article is well written and points out nicely the differences to the bacterial systems. However, it has a strong bias on the work that the Danish laboratory performed. Below I have made detailed comments, including those for missing references.

- Line 31: should be crRNA-DNA.
- Line 53: typo: cassette.
- Line 57: genetic tools developed in the Whitaker/Albers/Schleper labs should be cited here.
- Line 59: Bolotin *et al.* 2005 should also be cited here (Microbiology. 2005 Aug; 151(Pt 8): 2551–2561.)
- Line 68: variety of morphotypes—is a repetition of the sentence before.
- Line 69: citation 14 does not fit here (only haloarchaea), citation 15 is not a published manuscript.
- Line 74: citation 17 for unpublished data needed (?).
- Line 92: citation for this missing (Makarova *et al.* 2011, Koonin *et al.* 2007).
- Line 116: rephrase to: between the interference systems of type I and type III (not mechanisms).
- Line 126: should be: is only found in some members (delete only).
- Line 132–136: this sentence does not make sense to me.
- Line 138: reference Zhang *et al.* 2012 missing here (is ref 90).

- Line 142–144: unclear. isn't this rather a hypothesis? in my view should be presented more hypothetical.
- Line 155: why only in adaptation?
- Line 158: is shown (not are shown), delete: see below.
- Line 159–161: please rephrase.
- Figure 2: is the histogram really important to show?
- Line 168: never mentioned PAM before, write full name.
- Figure 3: why do we have to read the alignment from right to left?
- Figure 4: explain gray shading.
- Line 207: host genomes (not genome).
- Line 221: Pie plots (not pie-plots).
- Line 222: should be : to different classes of genetic elements.
- Line 231: citation No. 55 is not correct here. either remove citation or rewrite sentence.
- Line 233–242: there are also studies on the Cas6 of *S. solfataricus* that should be cited! (Sokolowski *et al.* 2014, Reeks *et al.* 2013, Lintner *et al.* 2011).
- Line 259: citation No.62 - looks like it does not belong here.
- Line 285/286: very speculative (involving cas1).
- Line 303: one should maybe mention casposons here.
- Chapter 5: very extensive chapter, could be kept shorter, is very detailed for *S. islandicus*.
- Line 331: introduce pMGB1 (not everybody is familiar with it, .. conjugative plasmid).
- Line 337: a conclusive sentence is missing for this paragraph.
- Line 359: should be: little is known about the mechanism...
- Line 375: word missing?
- Line 397: highly speculative.
- Figure 5: is LD 8.5 the same as LD 9 in Figure 5?, should be labeled the same way.
- Line 494: typo widely.
- line 490 to 491: I don't understand what is meant here!
- line 536–537: do we have a type IIIA system?
- line 557: CASCADE in capitals.
- Line 561/581/Figure 6: although Rouillon *et al.* is cited here, the csm (type IIIA) is missing in the description of the interference complexes (structure was solved).
- To my knowledge, there is no structure known for cmr-alpha—how can one predict such a scheme? I would rather prefer to see the CSM since the structure is solved.
- Line 636: should be Figure 7, not 6.
- Figure 7: why are the *att* sequences shown, are they of importance?
- Line 661: ref. 110: not clear to me, that the virus rearrangements of HAV1 come from CRISPR targeting, since no spacer was found to match the virus (only on translated level, which does not make sense to me).
- Line 680–681: this statement is contradictory to the one in the following paragraph.
- Line 725–729: it doesn't really make sense to me. at least one would have to mention that *S. acidocaldarius* has a cmr complex.

- Line 733: antisense RNA can be of variable length. It could also be that some do not carry a full repeat.
- Line 784: non-core.
- Line 802: another important question would be if CRISPR is involved in host gene expression, since some spacers have significant matches to host genes.

Round 1: Author Response to Reviewer 1

All the suggestions for minor clarifications, or other modifications, have been made in the text. All suggestions for additional references, or alternative locations for reference numbers, have also been followed.

Other points:

- The leader alignment from right to left in Figure 3 is because the leader lies upstream from the CRISPR locus and therefore starts at -1 (as for transcriptional leaders).
- We have not reduced the length of Section 5 because all the points raised are salient and fairly concise.
- We have now added Type III-D to Figure 6 as requested (and inserted a new version of the Figure in the text). We have also justified including a mixture of crystal structures and, by homology, predicted/speculative structures, so there should be no confusion for the reader.
- In Section 7.3, the host(s) of HAV1 are unknown, so it is just speculated that CRISPR defence could have produced the changes observed.
- The apparent contradiction in Section 7.4 is not a contradiction and is now clarified further.
- The *att* sites in Figure 7 are now referred to in the text.

Round 1: Reviewer 2 Report

Roger Garrett *et al.* review CRISPR-Cas systems found in *Sulfolobus* species. This is an accurate and detailed description of this rather specific field of research and the authors are leading experts on this topic. The manuscript is well-written and I have only minor comments that need to be addressed:

- (1). The authors should attempt to consider a more general audience that might not be familiar with basic CRISPR-Cas functionality details. Many abbreviations, like CRISPR and Cas, are not introduced. The terms “PAM” and “Cascade” are not explained. The beta of Type III-Bbeta is not explained.
- (2). The references and discussion are not always up-to-date in sections that concern CRISPR systems outside the Sulfolobales. This includes e.g. data available for the cleavage specificity of Type III systems.
- (3). Some polishing is left to do. Several track changes marks are left in the manuscript. Some other minor errors need to be fixed (e.g. prime vs. apostrophe, widely (page 16), CASCADE (page 9) vs. cascade (page 18), CasHD (page 9)). Section 5.7 contains three successive uses of “moreover”. Figure 2 overlaps with the figure legend.
- (4). It is not clear what grey/pink/blue boxes indicate in Figure 4.

- (5). The protein locations of some interference complexes in Figure 6 are termed speculative. What is this speculation based on? Might it be better to skip these?

Round 1: Author Response to Review 2

Some of the points overlap with those of referee 1 and all 5 points have been changed/clarified in the text, including defining the main scientific abbreviations.

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