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Study the Effects of Water Content on CAHS Proteins and its Vitrified Characteristics

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ABSTRACT: 29 bacterial isolates from the family Bacillaceae were taken from the International Space Station, assembled, and had their draught genome sequences analysed. We can better grasp these sequences' relevance in biotechnological and space applications through further investigation.

KEYWORDS: CAHS proteins, Long-term Spaceflight, Micro biome, Gut Brain Axis.

I. INTRODUCTION

Thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) are used to examine residual water in the CAHS protein RvCAHS1 and two species of tardigrades by Arakawa and Numata (2021) in their Letter, "Reconsidering the "glass transition" hypothesis that intrinsically unstructured CAHS proteins contribute to desiccation tolerance of tardigrades," in this issue of Molecular Cell.Please take note that the editor at Molecular Cell requested that an- imal studies not be included in this response, which is limited to in vitro experiments and a reexamination of Arakawa and Numata's data due to the need for a prompt response and the difficulty in obtaining the food source for R. varieornatus from Arakawa due to an export restriction.

II. WATER CONTENT ON CAHS PROTEINS

The first claim made by Arakawa and Numata is that other materials that aren't typically thought of as protective during desiccation can also vitrify, negating the idea that protection can be inferred from vitrification. Although we agree with this assertion, we are unable to see how restating it advances or undermines the conclusions we reached in Boothby et al. (2017). Additionally, contrary to conventional wisdom in the area, in our initial publication we did not hypothesise protection against vitrification but rather observed vitrification and conducted experiments to objectively link vitrification with survival (Sakurai et al., 2008). Additionally, we would want to draw attention to the fact that some of the molecules identified by Arakawa and Numata as vitrifying but that "clearly do not contribute to desiccation tolerance," such BSA, have been demonstrated to be protective during drying (Boothby et al., 2017, Piszkiewicz et al., 2019). Second, despite the fact that CAHS 77580 was seen to vitrify, Arakawa and Numata contend that neither RNAi nor heterologous expression studies demonstrated that this protein is required nor sufficient for desiccation resistance. It is likely that CAHS 77580 RNAi was just less effective than RNAi targeting other CAHS genes that did exhibit loss of tolerance, though, as RNAi is not entirely penetrant. Additionally, a cautious approach was used to determine the importance of functional as-says, and any results with a p value greater than 0.01 were deemed not significant (Figure 4 of Boothby et al., 2017). Most people would read the p value for the RNAi knockdown of CAHS 77580, which was $p > 0.01$, but $p 0.05$, as evidence that this protein is actually required for strong desiccation tolerance in tardigrades. This is in keeping with conventional judgments of significance. It is not proof that vitrification is unimportant because heterologous expression of CAHS 77580 in yeast led to an unique glass transition but not a statistically significant increase in desiccation tolerance. A vitrifying molecule's capacity to offer protection could be impacted by additional factors including expression level. Trehalose's protective properties, for instance, depend on vitrification, but levels of protection also depend on trehalose's intracellular concentration (Tapia et al., 2015). Similar to this, CAHS protection in vitro exhibits a pronounced concentration dependence (Boothby et al., 2017, Piszkiewicz et al., 2019), which probably also holds true in vivo. In conclusion, it is incorrect to conclude that CAHS proteins do not operate via vitrification based on findings from functional investigations in Boothby et al. (2017). Third, Arakawa and Numata contend that only lingering water is responsible for the reported glass transitions of CAHS proteins at 60°C. It's known that vitrified materials can contain water, and residual water remains in anhydrobiotic organisms even after drying (Clegg, 1978, 1986; Potts, 1994). (Blasi et al., 2005) In their RvCAHS1 samples maintained at ambient temperatures, Arakawa and Numata identified two thermal features: a "lower" (80°C) and a "higher" (160°C) characteristic. The

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lower thermal characteristic of the protein changes after drying in RvCAHS1 samples kept in the presence of silica drying agent, while the higher feature vanishes. According to their letter, the removal of this higher feature occurred because "the transition temperature moved over the recorded range." As demonstrated in Farahnaky et al. (2005), "an increase in the transition temperature Tg with lower starting water content is in keeping with earlier data. The authors may have missed this up-shift in their lower feature since it is partially obscured due to the original y axis scaling of their thermo- gram, but it is clearly visible when the y axis is rescaled (Figure S1B). This is consistent with the claim that CAHS pro- teins vitrify (Boothby et al., 2017). Due to the thermogram's initial y axis scaling, the authors may have missed this up-shift in the lower feature, but when the y axis is rescaled, it becomes very obvious (Figure S1B). This supports the idea that CAHS proteins vitrify (Boothby et al., 2017). We chose to further investigate the vitrifying properties of CAHS proteins by introducing humidity back to dried proteins, despite the fact that these up-shifts in Tg with drying are already indicative of vitrification. Humidity should have the reverse effect, causing vitrified materials to plasticize or weaken and a downward shift in Tg (Farahnaky et al., 2005). In order to measure Tg, we incubated duplicate samples of purified CAHS-D from Hypsibiusexemplaris at relative humidity (RH) levels of 60%, 70%, and 95% for the duration of an overnight vacuum concentrator drying process. As the humidity increased for these partially hydrated samples, the Tg clearly decreased, which is consistent with vitrification of the CAHS proteins (Blasi et al., 2005; Farahnaky et al., 2005), but not if water alone were solely responsible for the thermographic features. Furthermore, two unique thermal characteristics were seen in one 95% RH replicate, which was probably caused by the CAHS protein's uneven rehydration in that sample (Figure S1C). The assumption that these features are caused solely by water is in opposition to the presence of two unique thermal features because one would anticipate that water evaporation would occur across a single range, not two distinct temperatures. These new findings lead one to the conclusion that CAHS proteins vitrify in vitro and that CAHS DSC characteristics are not only attributable to water. Fourthly, according to Arakawa and Numata, the difference between the Tg of dried tardigrades and CAHS proteins shows that these proteins are not causing vitrification in tardigrades. This may sound obvious, however studies have shown that environmental factors like co-solvents and humidity have an impact on a material's glass transition temperature (Blasi et al., 2005; Farahnaky et al., 2005; Kasapis et al., 2003). Arakawa and Numata's fourth claim, which states that CAHS proteins are not necessary for tardigradevitrification, is untrue because one would not anticipate a pure protein in a simple buffer to have the same Tg when vitrified in a complicated cellular milieu. Fifth, according to Arakawa and Numata, noise is to blame for the 98C characteristic in the thermogram of preconditioned tardigrades shown in Boothby et al. (2017). Arakawa and Numata's data were not replicated, and they do not explain why their data lacks other high-temperature features present in Boothby et al. (2017) that are obviously not the result of noise. It is true that these data were not replicated due to the large input of animals required for such studies. The differing drying techniques used by Arakawa and Numata or their failure to consider animal survival as a factor are also potential sources of discrepancy. Moreover, Arakawa and Numata do not explain why their tardigrade DSC data did not confirm a separate study on tardigradevitrification, which found vitrification in 6 different tardigrade samples with Tgs that closely matched the 98-C Tg Boothby et al. discovered (for example, 92.9-C 5.3-C and 91.8-C 9.4-C). Given that (1) their data is not replicated, (2) a survival control was not carried out to ensure that animals without an observable Tg are desiccation tolerant, (3) their data did not replicate higher temperature DSC features that are obviously not due to noise, and (4) their data did not replicate higher temperature DSC features, it is unclear why the reader should accept Arakawa and Numata's conclusion that tardigrades are not vitrifying when anhydrobiotic. They were unable to reproduce findings from an independent investigation that discovered Tgs in a variety of tardigrade species that were comparable to those found in Boothby et al. (2017).

III. CONCLUSION

In conclusion, the tardigrade DSC findings of Arakawa and Numata do not provide a convincing refutation to several reports that tardigrades vitrify. Finally, Arakawa and Numata hypothesise that tardigrade desiccation tolerance may be related to the "high affinity of CAHS proteins to water." Given that their TGA data reveal that desiccation-tolerant tardigrades had less water by mass than desiccation-sensitive tardigrades, it seems unlikely that CAHS proteins help tardigrades retain water and that this is protective. As a matter of fact, their findings imply that tardigrades expressing CAHS proteins retain less water than those that do not. The five claims made in Arakawa and Numata's letter do not prove that tardigrades or CAHS proteins cannot vitrify upon desiccation, and their TGA results indicate that their proposed mechanism of water retention by CAHS proteins is doubtful. Although there is evidence that CAHS proteins vitrify, the vitrification hypothesis does not preclude the existence of other desiccation tolerance mechanisms.

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Trehalose mediates desiccation tolerance in Polypedilumvanderplanki larvae by vitrification and water replacement, according to research by Sakurai et al. (2008). Similar to the anchorage model, which postulates that a highly viscous or vitrifying material could help to coordinate leftover water molecules to enable desiccation-sensitive proteins to remain solvated and properly folded, it is possible that the vitrified matrices created by CAHS proteins may work in concert with what water is left (Bellavia et al., 2011). It will be fascinating to learn what fresh research reveals about additional defense-mediated CAHS protein pathways.

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