

Biogeosciences Discuss., referee comment RC2
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Comment on bg-2021-76

Takashi Toyofuku (Referee)

Referee comment on "Fluorescent double labelling of F-actin in Foraminifera: evaluation of granular pattern F-actin organisation in reticulopodia" by Jan Goleń et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-76-RC2>, 2021

General comment

This research investigated a point that was unclear in the previously published research. Although the content is good, it is questionable whether the purpose and discussion match the common interests of the audience of this journal. In addition, I think many points can be discussed with previous studies, but they are lacking. If these points are improved, I can agree there is a great possibility that the contents of this paper can be published in Biogeosciences.

L 21 Introduction

"...To account for this possibility, the term SiR-actin labelled granules has been coined to describe them (Goleń et al. 2020). The presented study primarily addresses the question, whether they are experimental artefacts or they represent physiological and functional forms of F-actin in foraminifera."

This targeting is too specific and does not contribute to the general readership.

Too much value is placed on the biological perspective for this study. It will be classified as a more biological and protozoan work. The discussion should return to the proposition that "Foraminifera pseudopodia are very important for understanding the evolution, morphogenesis, physiology, and ecology of these organisms." Otherwise, the position of this research in Biogeoscience and Earthscience will be unclear. It is essential to discuss this research and insights into biomineralization and shell morphology. Considering that cytoskeletal variation governs pseudopod extension and that the three-dimensional structure of the pseudopod unfolding from the aperture governs shell morphology (Tyszka et al, 2005; Tyszka, 2006), it should not be too difficult to connect and discuss the results of this study with these perspectives.

Method

L90.

If possible, can the origin of the samples be shown? I would imagine, however, that foraminifera would have been introduced mixed in with corals and other macro-organisms from various origins. Since authors can not know where they originated, it seems like a good idea to identify the species or provide SEM photos. I do not think there is any need to hesitate on account of the deformity. From my own research experience, I am aware that the shell morphology of Miliolid is easily affected in captive environments.

L.128

Add "Digital Single Lens Reflex camera" around Cannon DS 126231

L. 133

3.3 Control for autofluorescence □3.1 Control for autofluorescence

L. 140

"The staining of reticulopodium with both of fluorescent probes was successful "

Describe and discuss the reasons and conditions for what authors would call a "successful." Just quoting Figure 1-3 is not enough explanation, and the reader will not know if it is successful or not.

L. 334

Figure 1□Figure 2

L. 145

"Stanley 1971"

This study focuses on the localization identity, and it is a fundamental issue of this study, then needs a solid evaluation, rather than discussing the possibility ("possibly" in L144).

If this can be technically corrected, and it can be proved that there is no problem, then it is better to state in the method "analyzed by correcting for differences in depth of focus depending on wavelength" and describe the correction methodology in the supplement.

This study focuses on the localization identity, and it is a fundamental issue of this study.

L. 159

3.3 MT

Abbreviations that appear for the first time should be accompanied by an explanation.

L161

"The fact that three independent methods indicate presence of the F-actin ..."

What are the three methods, SiR-Actin, Phalloidin Atto 488, and birefringence? I believe that the authors have shown that they existed in the same place. However, it is a leap to say that this is the basis for showing that F-actin is present in the reticulopodium. Authors need to explain it sequentially to complete the logic for example.

The signal of SiR-Acrin was detected orthotopically with that of Phalloidin Atto 488. Phalloidin is known to bind specifically to F-actin and is used as a major indicator substrate for F-actin. (Cooper, 1987 DOI: 10.1083/jcb.105.4.1473). It is also known that F-actin bundles exhibit birefringence. (Hodge AJ. J Biophys Biochem Cytol. 1955 Jul 25;1(4):361-80. DOI: 10.1083/JCB.1.4.361; Hodge AJ. J Biophys Biochem Cytol. 1956 Jul 25;2(4 Suppl):131-42. DOI: 10.1083/jcb.2.4.131. These results strongly suggest that the signal of SiR-Actin indicates F-actin.

The above is an example, but this is the kind of discussion that needs to be addressed. This is the primary purpose of this paper. Further, TEM images of granular materials are also demanded in future studies. After that, we can start to discuss whether granular materials have much F-actin or not.

L. 195 "suggest that they are key evolutionary adaptation that most likely predated emergence of foraminiferal tests in the early Palaeozoic."

The results of Pawloski et al. (2013: already referred to in this study) should be cited and discussed. It is also essential to compare the results with Habura et al. (2005: <https://doi.org/10.1093/molbev/msi190>), who attempted to explain the quick movements of pseudopodia from the aspect of Tubulin.

L. 196 "They probably facilitate efficient formation of tests and fast reorganization of pseudopodial structures in Foraminifera. "

Provide evidence for why authors think so, and discuss the connection to shell formation.

L. 198 cannot be determined without more detailed ultrastructural studies

I agree with this statement, but there are many examples of previous studies that have observed the movement, function, and microstructure of pseudopods during shell formation. Based on the present findings, a discussion of the contents of these previous studies must be made. In particular, I believe that comparisons with and interpretations of the authors' previous studies can be made with certainty.