

## ***Interactive comment on “Dynamics and organization of actin-labelled granules as a rapid transport mode of actin cytoskeleton components in Foraminifera” by Jan Goleń et al.***

**Takashi Toyofuku (Referee)**

toyofuku@jamstec.go.jp

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### General comments

This study used a fluorescent probe "SiR-actin" that specifically stains actin filament. The authors describe whether or not it is actin with various potential pieces of evidence. Further, the authors recorded the behaviour of stained materials. In particular, they describe the movement of particles that are present on the pseudopodia. The particles were transported rapidly on the pseudopodia. The authors are assuming that it is a "packet of actin filament". It is hypothesized that it is one of the causes for the pseudopodia of foraminifers to be rapidly extendable and retractable. I like this series

C1

of observations and estimations from a general point of view. The content is complementary to Tyszka et al. (2019), which was previously published on ProNAS.

The results are presented in beautiful photomicrographs and are ambitious scientific manuscripts with new suggestions. I'm positive about this manuscript, but there are some points that I would like authors to improve for publication.

They suggest that what is stained in SiR-actin were the membranous surfaces of pseudopodial structures, linear or ring-like structures, and small but strongly labelled granular structures. Then, they defined these small but strongly labelled granular structures as actin-labelled granules (ALGs). The behaviour of ALGs is partially documented.

Furthermore, since mitochondria are known to distribute on the pseudopodia, they distinguish ALGs from mitochondria by Mitotracker green. Mitochondria should be indicated by Mitotracker green and not by SiR-actin. Then, the authors deny the possibility that ALGs are mitochondria. That is the reason why particles stained with SiR-actin present on the pseudopodia are not mitochondria but actin.

In interpretation and argument, it is claimed that the materials stained with SiR-actin are actin, this would be over-interpretation. Since this point is the limit of the fluorescent staining method. Argue the certainty from the comparison of the current results with the previous studies, TEM, and the fact that the distribution with mitochondria does not overlap. I would like you to discuss this point clearly and collectively in section 4.1. The possibility of the existence of the structure observed in the TEM of the previous studies should be included in this paragraph. If the authors will not discuss reliability and robustness in 4.1, readers cannot consider the following argument.

Description of the methodology that can be reproduced experimentally is essential for the scientific paper. Basic information such as what you observed with the filter set is missing in this study. The authors need to improve the writing of methodology. This point is the most unacceptable problem in this manuscript.

C2

It is overestimated and misleading to call those stained with SiR-actin as actin-labelled granules (ALGs).

Why not describe the structures stained with SiR-actin other than granule-like structures? I think it's as interesting as granules, so be sure to discuss it.

Discussions about evolutionary analysis (P12 L1) cannot be argued without actin live imaging in other taxa.

#### Questions and Comments

P1. L26 Correlative fluorescent.... It is not done in this study. Is the description necessary in the abstract?

P3. L20 Describe the exact number of used species in this study.

P4. L14 How long? How about food material? What will be labelled by calcein-AM with calcium-free seawater?

P4. L16 Categorize by purpose, not the institute.

P4. L16 Refer to Ohno, Y., et al. "Cytological Observations of the Large Symbiotic Foraminifer *Amphisorus kudakajimensis* Using Calcein Acetoxymethyl Ester (vol 11, e0165844, 2016)." PLOS ONE 12.4 (2017).

P4. L17 remove ")"

P4. L19 Indicate the excitation and emission wavelengths of all probes.

P4. L20 " it is not possible to use this probe to label F-actin within the endoplasm" The authors show the SiR-actin fluorescent in the cell (Fig. 4). Explain exactly. In fact, it is difficult to distinguish between signals and autofluorescent from chlorophyll.

P4. L22 The descriptions are not enough to reproduce the experiment. How did authors decide experimental/observation settings (e.g. excitations/emissions and exposure times) of each probe? The settings of conventional optical observation should

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be indicated, too. Were there some negative controls? What was the frequency of time-lapse imaging?

P4. L22 Indicate the setting of fluorescent cubes.

P4. L30 " tripled in some images." Did such images used for analyses? What criteria do you use to decide whether to use images or not? How many shooting cases are there?

P5. L5 Be sure to indicate whether each image is by laser confocal or by ApoTome.

P5. L6 Rather than being confirmed to be actin, it is used in the sense of particles stained with SiR-actin. Is it not misleading?

P5. L8 Put some description of DIC observation in Materials and Method.

P5. L11 This text contradicts that the cytoplasm has symbiotic algae and is unobservable.

P5. L12 Had authors mixed and discussed the results of different species? Are there variations among species?

P6. L1 Isn't "SiR-"actin-labelled granules?

P6. L3 Show the variation of dynamics. Can you make a summarized table?

P6. L6 It is a good approach.

P6. L15 Although it is difficult to measure the dynamics of the granule, the authors observe the movement of the fluorescently labelled granules that were seen along the pseudopod. This makes it possible to observe the movement of granules by limiting into a one-dimensional movement. For example, if time is plotted on the horizontal axis and the coordinates in the pseudopodia on the vertical axis, can it be possible to illustrate temporal changes in granule's position.

P6. L19 How did you calculate the rate of granule movement? Did you show the

C4

measurement method in the materials and methods?

P6. L22 Indicate the dynamics of other types of a granule.

P7. L5 FLAKOWSKI et al can be found in the reference list. I guess the authors refer to the study here. Discuss the relationship between foraminiferal actin variability and phylogeny.

P7. L18 "Such effects have not been reported." Did you compare the results with the population of negative controls?

P8. L10 Please reconsider the subtitles. It does not match the content.

P8. L16 This paragraph plays the role of the just introduction of following chapters not a discussion of results. Reconstruct the chapter structure. e.g. The chapter number 4.4 should be 4.3.1.

P10. L9 4.5.1 is numbered twice.

P10. L13 "representstructurally" insert space.

P21. L3 What does the cloudy distribution of SiR-actin around endosymbiont? No signal was detected in the same region by calcein-AM.

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