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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.

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As someone who has studied cytoskeletal structure and function for many years in various cell types, I was excited to see new information regarding the distribution of f-actin in foram reticulopodia! The paper represents a logical extension of research done on this topic primarily in the 1980s and 1990s. Using confocal light microscopy and a different fluorescent probe (SiR), the authors describe f-actin distribution in several pseudopodial morphotypes in a number of foram species. In this regard, the work represents a novel contribution.

The results presented are interesting, but the author's conclusions can only be consid-

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Discussion paper

ered hypothetical at this point. Of paramount importance is the correlation of fluorescence light microscopy images with electron microscopy; the simple comparisons with published photographs used here are not at all convincing. The authors should be obligated to show directly what the staining patterns correspond to ultrastructurally. (There are many straightforward ways to do this.) To be more complete, it would also be desirable to illustrate motile events (granule motion, etc.) immediately prior to fixation for electron microscopy.

Critical controls, missing from the present study, include demonstrating that the observed SiR staining patterns are not caused by the action of jasplakinolide. The authors (and sales literature) suggest that they are not, but to examine this important issue experimentally the authors should fix the cells first and then stain for f-actin using SiR and fluorescent phalloidin; equivalent patterns using two independent f-actin probes in fixed cells would be much more convincing. An important allied question is: what is the effect of unlabeled jasplakinolide on f-actin distribution and reticulopodial motility? Such information would help flesh out their study and provide important new information on the pharmacological disruption of foram cytoskeletal dynamics.

A storage form of f-actin? Because actin is *highly* abundant in eukaryotic cells, it would be remarkable for it to be transported as oligomers or filaments, as suggested. To make the claim believable, the authors would have to provide evidence that g-actin concentrations in reticulopods are insufficient to support localized assembly. (There is a vast literature on g-actin transport or storage forms of g-actin complexed with assembly regulatory proteins, in neurons, sperm acrosomes, etc., that the authors can consult to guide their work.)

As a final point, I question the "fit" for this study being published in Biogeoscience. It seems more suitable for a cell biology or protistology journal, where it will receive much more attention.

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