Lifeact cannot visualize some forms of stress-induced twisted f-actin

To the Editor: Reidl *et al.* have described the use of a small polypeptide, Lifeact, derived from yeast, to visualize filamentous actin (f-actin)[1]. This reagent, when fused to GFP, allows the study of actin remodeling in living cells without consequences to actin polymerization. The authors concluded that "Lifeact can be used as a universal marker for actin imaging..." [1].

We fused the Lifeact sequence to either monomeric (m)Cerulean blue[2] or mRFP[3] and expressed these proteins in mouse striatal neuron–derived STHdh cells to study the formation of nuclear cofilin-bound actin rods that form during cell stress (**Supplementary Methods** online).

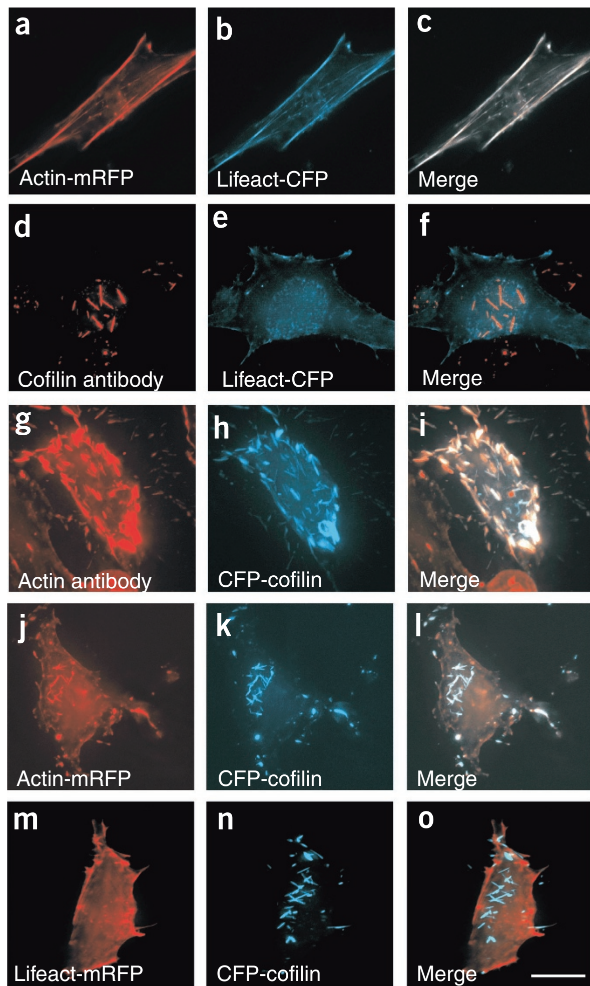

Figure 1 | Visualization of actin and cofilin in nuclear rods after stress. (**a**–**o**) Fluorescence images of STHdh cells transfected with the indicated fusion proteins or of endogenous proteins in STHdh cells detected with the indicated antibodies. Cells were grown at 37 °C without heat shock (**a**–**c**) or after 30 min heat shock at 42 °C (**d**–**o**). Merged red-cyan signal is pseudocolored white. Scale bar, 10 μm.

We found that Lifeact-CFP could be used to visualize f-actin under optimal growth at 37 °C, with Lifeact signal very similar to that of actin-mRFP (**Fig. 1a–c**). Upon cell stress induction (**Supplementary Methods**), we observed the formation of typical cofilin-bound actin rods in the nuclei of cells with endogenous amounts of cofilin by analyzing immunofluorescence of an antibody to cofilin (**Fig. 1d**). However, we did not detect actin in these structures with Lifeact-CFP (**Fig. 1e**,**f**), but Lifeact highlighted nuclear actin puncta (**Fig. 1b**,**e**). Using CFP-cofilin to localize the position of stress-induced rods, we detected the presence of actin in the nuclear or cytoplasmic rods either by immunofluorescence analysis (**Fig. 1g**) together with CFP-cofilin (**Fig. 1h**,**i**) or by actin-mRFP expression (**Fig. 1j**) together with CFP-cofilin (**Fig. 1k**,**l**). To test whether the fused fluorophore protein may be affecting Lifeact activity, we switched from using CFP to mRFP. Whereas Lifeact-mRFP could be used to visualize actin similar to Lifeact-CFP (**Fig. 1m**), we found that Lifeact could not be used to visualize nuclear rods that contain f-actin (**Fig. 1m–o**). Heat shock did not appear to affect the ability of Lifeact to recognize f-actin in cytoplasmic stress fibers (**Fig. 1e**,**m**).

Cofilin responds to cell stress by arresting actin dynamics, thus freeing cellular ATP[4]. Cofilin-bound actin rods persist after cell stress in Alzheimer's disease–affected neurons and can be induced by a variety of cell stresses[5]. Others have shown that cofilin-actin rods cannot be stained with fluorescent dye–labeled phalloidin, as cofilin binding to f-actin stabilizes the twisted state of f-actin in the rods, which prevents phalloidin binding[6]. The most parsimonious explanation of our results is that f-actin conformational changes upon cofilin binding prevent Lifeact from binding to twisted f-actin. To date, the only way we can accurately visualize actin in cofilin-actin rods in live cells is with the use of an *Aequoria* sp. fluorescent protein (AFP) fused to actin.

These data provide an important caveat for those using Lifeact and studying actin dynamics during cell stress and indicate that Lifeact may not be a universal tool for studying actin dynamics in live cells. These data also suggest that Lifeact may be a useful tool to distinguish certain forms of f-actin in live cells.

Note: Supplementary information is available on the Nature Methods website.

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