© 2017. Published by The Company of Biologists Ltd | Journal of Cell Science (2017) 130, 1688 doi:10.1242/jcs.204487

CORRECTION

Correction: Actin visualization at a glance

Michael Melak, Matthias Plessner and Robert Grosse

There was an error published in *J. Cell Sci.* **130**, 525-530.

Unfortunately, the diagram of the actin filament in the poster was accidentally reflected during the creation of the figure. As a result, the filament was illustrated as a left-handed rather than a right-handed F-actin helix in the original version of this article. The online version of the article has been corrected accordingly. This change has no impact on the concepts presented in this article.

The authors apologise to the readers for any confusion that this error might have caused.

Journal of Cell Science

© 2017. Published by The Company of Biologists Ltd | Journal of Cell Science (2017) 130, 525-530 doi:10.1242/jcs.189068

CELL SCIENCE AT A GLANCE

Actin visualization at a glance

Michael Melak, Matthias Plessner and Robert Grosse*

ABSTRACT

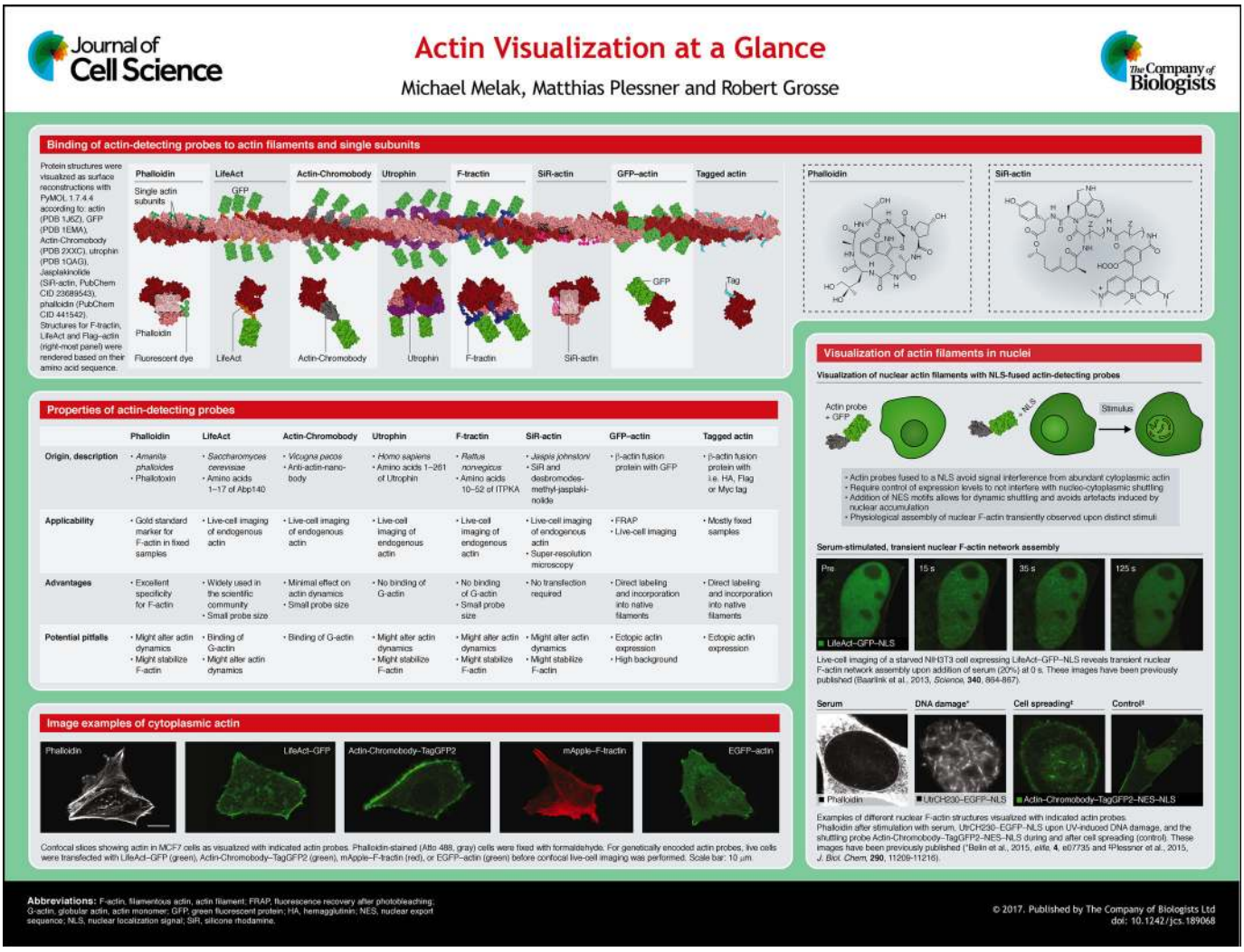
Actin functions in a multitude of cellular processes owing to its ability to polymerize into filaments, which can be further organized into higher-order structures by an array of actin-binding and regulatory proteins. Therefore, research on actin and actin-related functions relies on the visualization of actin structures without interfering with the cycles of actin polymerization and depolymerization that underlie cellular actin dynamics. In this Cell Science at a Glance and the accompanying poster, we briefly evaluate the different techniques and approaches currently applied to analyze and visualize cellular actin structures, including in the nuclear compartment. Referring to the gold standard F-actin marker phalloidin to stain actin in fixed samples and tissues, we highlight methods for visualization of actin in living cells, which mostly apply the principle of genetically fusing fluorescent proteins to different actin-binding domains, such as LifeAct, utrophin and F-tractin, as well as anti-actin-nanobody technology. In addition, the compound SiR-actin and the expression of GFP–actin are also applicable for various types of live-cell analyses. Overall, the visualization of actin within a physiological context requires a careful choice of method, as well as a tight control of the amount or the expression level of a given detection probe in order to minimize its influence on endogenous actin dynamics.

KEY WORDS: Actin dynamics, Actin probes, Live-cell imaging, Nuclear actin

Institute of Pharmacology, Biochemical-Pharmacological Center (BPC), University of Marburg, Karl-von-Frisch-Straße 1, Marburg 35043, Germany.

*Author for correspondence (robert.grosse@staff.uni-marburg.de)

R.G., 0000-0002-3380-5273



Journal of Cell Science

Journal of Cell Science (2017) 130, 525-530 doi:10.1242/jcs.189068

Introduction

Actin is one of the most abundant proteins in eukaryotic cells and its amino acid sequence is very highly conserved from yeast to man. Concentrations of actin in eukaryotic cells are well within the micromolar range (Wu and Pollard, 2005), and, hence, actin fulfills a multitude of cellular functions, such as intracellular transport, adhesion and contraction, membrane dynamics and migration, cytokinesis and cell–cell contact regulation, polarity and cell shape control (Dominguez and Holmes, 2011), as well as gene regulation (Louvet and Percipalle, 2008; Bunnell et al., 2011) and other less-well explored functions in the nucleus (Grosse and Vartiainen, 2013; Plessner and Grosse, 2015).

In contrast to microtubules, which typically form long and straight cylindrical polymers with an outer diameter of 25 nm (Wade, 2007), actin filaments (F-actin) are composed of two twisted helices with a diameter of ∼5 to 9 nm (Holmes et al., 1990). One intrinsic characteristic of single actin proteins is their highly dynamic turnover between a monomeric and a polymeric state, which can result in rapid filament assembly and disassembly.

Although methods for the visualization of single microtubules have been well established for decades (Sherwin and Gull, 1989; Kikkawa et al., 1994), it has been and still is a challenge to visualize specific actin structures and their related functions, in particular under real-time conditions in living cells and organisms. Consequently, many different approaches and probes have been developed over the past years to monitor the dynamics of actin assembly and actin filament structures in several different model systems, while mostly trying to illuminate endogenous actin proteins.

Recently, several publications have discussed in great detail the benefits and disadvantages of different fluorescent actin reporters (Belin et al., 2014; Spracklen et al., 2014; Lemieux et al., 2014; Du et al., 2015). In this short article, and the accompanying poster, we present an at-a-glance view of the current approaches to visualize actin filaments, emphasizing the advantages and pitfalls of the available tools to investigate F-actin not only in the cytoplasm, but also in the somatic cell nucleus.

Visualization of F-actin in fixed cells and tissues

Visualization of the actin cytoskeleton was initially performed by immunofluorescence techniques that used antibody staining against fixed actin structures (Lazarides and Weber, 1974). To overcome possible drawbacks of antibody-based labeling techniques, such as non-specific binding, actin scavenging, high background, or variations in the epitope among different species, additional actin-binding components with a high specificity for F-actin were developed.

The gold standard marker for labeling endogenous actin filaments in fixed samples still remains phalloidin, a fluorescently labeled derivative of the phallotoxin, which binds to F-actin with high affinity (Wulf et al., 1979; Vandekerckhove et al., 1985) (see poster). Usage of phalloidin is largely restricted to the labeling of fixed cells, as this toxin only has a low permeability for cell membranes and more importantly stabilizes actin filaments *in vivo* and *in vitro* (Coluccio and Tilney, 1984; Visegrády et al., 2004; Wehland et al., 1977). Thus, the actin structures visualized by fluorescent phalloidin are in most cases arrested or altered to a certain degree by the necessary fixation and permeabilization procedures and, therefore, the obtained results need to be interpreted with caution. Depending on the permeabilization and fixation protocol, formation of artifacts can occur. For example, it has been shown that methanol fixation methods are not to be suitable for actin staining with phalloidin, as it can destroy the native F-actin conformation, resulting in labeling of artificial structures (Kellogg et al., 1988). Another drawback of using fluorescent phalloidin to label endogenous F-actin in native cells and tissues is that it might not bind to all the actin structures present. For example, the binding of cofilin to F-actin has been shown to interfere with fluorescent-dye-labeled phalloidin staining due to alterations of the F-actin structure (Bunnell et al., 2011; McGough et al., 1997). Finally, at least seven actin subunits are necessary for successful binding of phalloidin to F-actin (Kristó et al., 2016), preventing labeling of short F-actin polymers with phalloidin.

Ectopic expression of epitope-tagged actin (i.e. Myc-, HA- or Flag-tagged actin) in combination with immunofluorescence staining has been widely used to study actin organization in cells (Copeland and Treisman, 2002; Miralles et al., 2003). This simple approach can be helpful, but only if the expression levels of the probe are carefully controlled and titrated. Clearly, overexpression of tagged actin variants can be problematic, as even a subtle change in the amount of global actin can interfere with the physiological actin dynamics, and could trigger actin polymerization by itself (Mounier et al., 1997; Ballestrem et al., 1998). In addition, this approach is not well suited for live-cell imaging owing to the necessary immunofluorescence staining, unless actin is labeled with directly stainable tags, such as SNAP- (Keppler et al., 2004; Lukinavičius et al., 2013) or tetracysteine tags (Griffin et al., 1998). For instance, actin fused to a tetracysteine tag can be directly visualized by using different labeling reagents, such as the biarsenical dyes FlAsH and ReAsH (Martin et al., 2005). The relatively small size of the tetracysteine tag (∼2 kDa) might not interfere dramatically with different functions of actin, although it has been shown that the fission yeast formin Cdc12p, which is required for formation of the contractile ring, excludes tetracysteine-tagged fission yeast actin, suggesting very stringent structural requirements for formins to promote the elongation of actin filaments (Chen et al., 2012). Moreover, owing to potential chemical toxicity caused by the labeling reaction, such as the accumulation of FlAsH in active mitochondria (Langhorst et al., 2006), this technique might be better suited for short-term live-cell imaging.

Live-cell imaging using GFP–actin derivatives

Tagging actin with GFP derivatives is extensively used for live-cell analysis. In particular, GFP–actin is suitable for fluorescence recovery after photobleaching (FRAP) approaches to study actin dynamics and turnover within a given cellular F-actin structure, including, for instance, the actin cortex (Clark et al., 2013) or protrusions such as lamellipodia (Koestler et al., 2009) (see poster). However, the relatively large size (∼28 kDa) of GFP-like proteins (Sliogeryte et al., 2016) can give rise to problems in terms of incorporation of GFP-tagged actin monomers into filaments. Thus, the GFP–actin-derived localization pattern might only reflect a part of the total F-actin network in cells. For example, it has been shown in yeast that GFP–actin does not incorporate effectively in a specific subpopulation of actin filaments (Doyle and Botstein, 1996). Furthermore, certain actin nucleators, such as members of the formin family, might exclude GFP–actin monomers from their nucleation and elongation mechanisms, probably due to steric hindrances during the formation of actin seeds or during efficient barbed-end actin incorporation (Wu and Pollard, 2005; Vavylonis et al., 2006; Carvalho et al., 2009).

In summary, as is the case for any genetically encoded protein tagging, the expression of GFP–actin needs to be accurately

Journal of Cell Science (2017) 130, 525-530 doi:10.1242/jcs.189068

controlled in any given cellular system. Nevertheless, GFP–actin has been proven to be highly useful to image actin functions in living cells or organisms, such as in mammalian cells (Ballestrem et al., 1998), yeast (Doyle and Botstein, 1996), *Dictyostelium discoideum* (Neujahr et al., 1997; Westphal et al., 1997), *Drosophila* (Verkhusha et al., 1999) and mice (Gurniak and Witke, 2007).

Imaging F-actin structures and dynamics using actin-binding domains

There are currently several probes available to visualize endogenous actin dynamics in living cells. With the exception of SiR-actin (see below), these are based on genetically encoded actin-binding domains (ABDs) that have been derived from yeast or human proteins, which are fused to GFP derivatives.

The most popular and widely used such tool is LifeAct, a short 17-amino-acid peptide from yeast Abp140 (Riedl et al., 2008). There are numerous publications describing the successful application of LifeAct, in particular in mammalian cell motility (Rullo et al., 2012; Grikscheit et al., 2015; Suarez et al., 2015; Duleh and Welch, 2012; Fiolka et al., 2012) (see poster). However, experimental and technical drawbacks in using LifeAct as an actin probe in living cells have also been described. For example, one disadvantage of LifeAct–GFP variants is their relatively high background fluorescence owing to their high affinity for globular actin (G-actin) (Riedl et al., 2008).

Furthermore, although LifeAct has been suggested to be a universal marker for actin (Riedl et al., 2008), it is unable to stain certain actin structures, including a specialized class of actin-based filopodia in mesenchymal cells of the developing limb bud of chick embryos (Sanders et al., 2013) and stress-induced cofilin-bound F-actin structures in mouse striatal neuron-derived STHdh cells (Munsie et al., 2009). Both studies suggest that extensive coating of actin filaments with actin-binding proteins, such as cofilin, result in conformational changes of F-actin, thereby preventing LifeAct from accessing its binding site. In addition, at higher expression levels, LifeAct can influence the formation of actin filaments. It has been reported that, in contrast to expression in somatic cells or weak expression in the germline, a strong expression of LifeAct in the *Drosophila* germline results in severe actin remodeling and fertility defects (Spracklen et al., 2014). Moreover, it has been shown that LifeAct affects actin assembly during endocytosis and cytokinesis in fission yeast (Courtemanche et al., 2016). Although the underlying mechanisms are not well understood, both studies claim that the LifeAct-mediated effects on actin dynamics are concentration-dependent and thus suggest that the use of LifeAct requires a more elaborate optimization process. Nevertheless, an important advantage of LifeAct is its relatively low toxicity when its expression level has been carefully optimized. This is reflected in the successful generation of transgenic animals, such as mice or zebrafish (Riedl et al., 2010; Mizoguchi et al., 2016; Phng et al., 2013; Schachtner et al., 2012). Furthermore, LifeAct has been successfully used for actin imaging in plants such as *Arabidopsis* (van der Honing et al., 2011; Vidali et al., 2009) and in several fungi (Berepiki et al., 2010; Delgado-Álvarez et al., 2010).

In addition to LifeAct, the tandem calponin homology domains (CH1 and CH2) of utrophin (UtrCH) are frequently used as a marker for stable and dynamic F-actin, both in living and fixed cells (Burkel et al., 2007). UtrCH comprises the first 261 amino acids of human utrophin, an actin-binding, dystrophin-related protein (Winder et al., 1995). It has been suggested that the two CH domains of Utr261 interact with adjacent actin subunits (Lin et al., 2011). As actin is highly conserved, UtrCH has been successfully used as a marker for actin filaments in a wide range of organisms and cell types, including mammalian, amphibic or echinodermal oocytes and embryos, without showing negative effects of UtrCH on both actin organization and dynamics (Burkel et al., 2007; Holubcová et al., 2013) (see poster). However, more severe defects related to actin dynamics have been reported, such as cortical actin breakdown and alterations in the morphology of actin bundles, as well as female sterility during *Drosophila* oogenesis, when UtrCH is expressed at higher levels (Spracklen et al., 2014).

Another tool, later on termed F-tractin, is a 43-amino-acid long peptide from the rat actin-binding inositol 1,4,5-trisphosphate 3-kinase A [corresponding to residues 10–52 (Belin et al., 2014)] (Schell et al., 2001) (see poster). F-tractin is less-well studied than LifeAct and thus there are fewer imaging studies using this probe. Because F-tractin is larger in size than LifeAct, it might interfere with other regulatory or accessory F-actin-binding proteins. For instance, F-tractin expression has been shown to alter the organization and morphology of the actin cytoskeleton in a mesoderm-derived cell line from *Xenopus* (Belin et al., 2014). However, in contrast to LifeAct and UtrCH, F-tractin has been reported to not perturb actin rearrangement during *Drosophila* follicle development (Spracklen et al., 2014), suggesting that it might be the most suitable actin probe to study F-actin dynamics in *Drosophila* nurse cells. However, further studies are necessary to fully evaluate the potential advantages of using F-tractin over the currently more frequently used imaging probes.

Furthermore, as mentioned above, some actin markers are excluded from specific actin filament structures, and therefore distinct actin probes might display a differential cytoskeletal architecture. An interesting recent study compared the distribution of GFP–actin, F-tractin, LifeAct and utrophin in different living cells (Belin et al., 2014) and found, for example, that actin-binding probes often insufficiently stain actin in filopodia-rich regions, whereas GFP–actin is incorporated well into filopodia. By contrast, GFP–actin is often excluded from stress fibers and lamellar filament structures, reflecting its incompatibility with other endogenous actin-binding proteins. Overall, the authors concluded that among all the analyzed fluorescent derivatives of actin and ABDs, F-tractin most accurately reproduced F-actin structures as they could be visualized by phalloidin labeling in a wide variety of cells (Belin et al., 2014).

More recently, the cell-permeable chemically synthesized probe SiR-actin, has been introduced (D'Este et al., 2015); it is structurally derived from the high affinity F-actin-binding toxin Jasplakinolide from the marine sponge *Jaspis johnstoni* (Bubb et al., 1994) (see poster). Advantages of SiR-actin are its ease of use in imaging approaches as it circumvents any time-consuming cell transfection and protein overexpression systems. However, caution must be taken in live-cell imaging as SiR-actin might cause F-actin stabilization or induce actin polymerization owing to its structural similarities to Jasplakinolide. This can make interpretations of observed F-actin structures more difficult, as they might have been assembled by the probe itself. Further studies are therefore needed to fully assess the advantages and possible limitations of SiR-actin over more established actin probes.

Visualizing actin dynamics using actin-directed nanobodies

A more novel approach to visualize actin filament assembly and disassembly in living cells utilizes single-domain antibodies, or nanobodies, which specifically bind to a singular antigen; these nanobodies are composed of a single domain and do not rely on a

Journal of Cell Science (2017) 130, 525-530 doi:10.1242/jcs.189068

quaternary structure as is the case for conventional antibodies. One example is the Actin-Chromobody (ChromoTek), a cameloid nanobody directed against actin that has been genetically encoded and fused to TagGFP2 (see poster). When expressed in cells, the Actin-Chromobody also produces a high level of background fluorescence, similar to LifeAct. However, in contrast to LifeAct, the Actin-Chromobody does not seem to influence apparent actin dynamics, even when it is expressed at high levels. So far, the Actin-Chromobody has been successfully applied to visualize actin in mammalian nuclei (Plessner et al., 2015), in tobacco leaf cells (Rocchetti et al., 2014) and zebrafish (Panza et al., 2015). Nevertheless, at present, it remains a novel and less-well explored tool.

Nuclear probes for monitoring F-actin assembly

Nuclear actin filaments and their specific functions are an emerging topic in the field of actin research. However, there are several reasons why the visualization of actin filaments in the nucleus is particularly difficult. First, the concentration of actin in the nucleus is much lower than in the cytoplasm (Baarlink et al., 2013). Second, the assembly of nuclear actin filaments is only triggered by specific signaling cues, for example in response to extracellular signals, such as serum factors (Baarlink et al., 2013) or DNA damage (Belin et al., 2015), or in the context of integrin-based cellular adhesion and mechanotransduction (Plessner et al., 2015). Finally, actin, as well as most of the actin-regulatory proteins found in mammalian cell nuclei, dynamically shuttles between the cytoplasm and the nuclear compartment (Kristó et al., 2016).

Staining of fixed nuclear actin filaments can be achieved by careful fixation with formaldehyde or glutaraldehyde, followed by phalloidin staining (Baarlink et al., 2013) and image acquisition with a state-of-the-art confocal microscope as well as a sensitive detection method (see poster). Live-cell imaging of nuclear actin requires the expression of actin-detecting probes that are fused to a nuclear localization signal (NLS) (see poster), such as the nuclear-targeted Actin-Chromobody–GFP (nAC–GFP) (Plessner et al., 2015). This helps to circumvent the comparably high signal of actin that emanates from the cytoplasm, and, indeed can be used to visualize nuclear actin filament assembly in living cells (Plessner et al., 2015). However, it is important to note that a compartment-specific localization of actin-detecting probes might influence the shuttling dynamics of actin and actin-binding proteins. Thus, we recently generated modified nuclear actin probes that also contains an additional nuclear export signal (NES) (LifeAct–GFP–NLS–NES and Actin-Chromobody–TagGFP2–NLS–NES), which allow for dynamic nucleocytoplasmic shuttling of the probe itself and so additionally enable the simultaneous visualization of cytoplasmic and nuclear actin structures (Plessner et al., 2015).

It is, therefore, important to avoid artificial transient overexpression to ensure that the probe does not interfere with nuclear actin dynamics (Du et al., 2015). In general, usage of stably expressing cell lines or viral expression systems might be better suited to avoiding artifacts caused by genetically encoded actin probes.

Conclusions and perspectives

A number of approaches and probes have been generated to visualize actin and the many types of filamentous structures it generates. The approaches and tools discussed here each have their advantages and pitfalls, and some of the tools are currently more commonly employed than others. One limitation of genetically encoded actin probes as well as phalloidin is their inability to distinguish between specific actin isoforms. Antibody-based approaches have been developed to address this (Perrin and Ervasti, 2010).

A major reason to decide for or against a particular actin-detection probe is the cellular context under study. Using the example of LifeAct, it has been proposed to be the most suitable actin probe to visualize the dynamic rearrangement of the actin cytoskeleton in *Dictyostelium*, as it labels a more complete subset of actin structures than other actin-binding probes (Lemieux et al., 2014). The application of LifeAct was also favored over actin fused to fluorophore in the context of visualizing actin rearrangements during cellular mechanotransduction events (Sliogeryte et al., 2016; Deibler et al., 2011). In contrast, LifeAct should be used with caution for actin visualization in fission yeast, as it can cause severe concentration-dependent defects in endocytosis and cytokinesis (Courtemanche et al., 2016). Furthermore, the suitability of the actin probe UtrCH is also highly dependent on the model organism or cell line. For example, UtrCH has been successfully used to visualize actin dynamics in mouse (Schuh, 2011; Holubcová et al., 2013) and *Xenopus* oocytes, as well as in several species of *Echinodermata* (Burkel et al., 2007). In contrast, a strong expression of UtrCH in the *Drosophila* germline has been reported to disrupt reorganization of the actin cytoskeleton during *Drosophila* oogenesis (Spracklen et al., 2014).

In addition, the choice of an appropriate fluorescent protein to be fused to an ABD is also of importance. For instance, a recent publication has demonstrated that the subset of F-actin that is recognized by diverse ABDs can be altered by the fluorescent protein or the linker sequence between the ABD and the fluorescent protein (Lemieux et al., 2014). New applications for fluorescent labeling of proteins have become available recently, such as the use of Y-FAST, a small monomeric protein tag enabling reversible binding and activation of a cell-permeant and nontoxic fluorogenic molecule (Plamont et al., 2016), or the flavoprotein improved LOV (iLOV), a fluorescent protein based on the light, oxygen or voltage domain (LOV) from a variety of sources (Buckley et al., 2015). Both molecules offer advantages over GFP and other related fluorescent reporters owing to their relatively small size. Therefore, they might provide valuable alternatives for actin visualization when fused to actin itself or to ABDs.

In summary, every detection method has limitations and might alter endogenous actin dynamics to various degrees. Hence, the choice and usage of any given probe has to be carefully considered and evaluated in the cellular system under study to be able to achieve an optimal reflection of the actin structure of interest within its physiological context. It is therefore important to validate obtained results with several probes to minimize any potential non-physiological observations or formation of artificial F-actin aggregations caused by the probe itself.

Acknowledgements

We thank members of our laboratory for helpful discussions. We thank J. Ivaska for providing mApple-F-tractin.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was supported by grants from the Deutsche Forschungsgemeinschaft (GR2111-7-1) and the Wilhelm Sander-Stiftung (2013.149.1) to R.G.

Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.189068.supplemental

References

Baarlink, C., Wang, H. and Grosse, R. (2013). Nuclear actin network assembly by formins regulates the SRF coactivator MAL. *Science* **340**, 864-867.

Ballestrem, C., Wehrle-Haller, B. and Imhof, B. A. (1998). Actin dynamics in living mammalian cells. *J. Cell Sci.* **111**, 1649-1658.

Belin, B. J., Goins, L. M. and Mullins, R. D. (2014). Comparative analysis of tools for live cell imaging of actin network architecture. *Bioarchitecture* **4**, 189-202.

Belin, B. J., Lee, T. and Mullins, R. D. (2015). DNA damage induces nuclear actin filament assembly by Formin-2 and Spire-1/2 that promotes efficient DNA repair. [corrected]. *Elife* **4**, e07735.

Berepiki, A., Lichius, A., Shoji, J. Y., Tilsner, J. and Read, N. D. (2010). F-actin dynamics in Neurospora crassa. *Eukaryot. Cell* **9**, 547-557.

Bubb, M. R., Senderowicz, A. M., Sausville, E. A., Duncan, K. L. and Korn, E. D. (1994). Jasplakinolide, a cytotoxic natural product, induces actin polymerization and competitively inhibits the binding of phalloidin to F-actin. *J. Biol. Chem.* **269**, 14869-14871.

Buckley, A. M., Petersen, J., Roe, A. J., Douce, G. R. and Christie, J. M. (2015). LOV-based reporters for fluorescence imaging. *Curr. Opin. Chem. Biol.* **27**, 39-45.

Bunnell, T. M., Burbach, B. J., Shimizu, Y. and Ervasti, J. M. (2011). Beta-Actin specifically controls cell growth, migration, and the G-actin pool. *Mol. Biol. Cell* **22**, 4047-4058.

Burkel, B. M., Von Dassow, G. and Bement, W. M. (2007). Versatile fluorescent probes for actin filaments based on the actin-binding domain of utrophin. *Cell Motil. Cytoskelet.* **64**, 822-832.

Carvalho, A., Desai, A. and Oegema, K. (2009). Structural memory in the contractile ring makes the duration of cytokinesis independent of cell size. *Cell* **137**, 926-937.

Chen, Q., Nag, S. and Pollard, T. D. (2012). Formins filter modified actin subunits during processive elongation. *J. Struct. Biol.* **177**, 32-39.

Clark, A. G., Dierkes, K. and Paluch, E. K. (2013). Monitoring actin cortex thickness in live cells. *Biophys. J.* **105**, 570-580.

Coluccio, L. M. and Tilney, L. G. (1984). Phalloidin enhances actin assembly by preventing monomer dissociation. *J. Cell Biol.* **99**, 529-535.

Copeland, J. W. and Treisman, R. (2002). The diaphanous-related formin mDia1 controls serum response factor activity through its effects on actin polymerization. *Mol. Biol. Cell* **13**, 4088-4099.

Courtemanche, N., Pollard, T. D. and Chen, Q. (2016). Avoiding artefacts when counting polymerized actin in live cells with LifeAct fused to fluorescent proteins. *Nat. Cell Biol.* **18**, 676-683.

Deibler, M., Spatz, J. P. and Kemkemer, R. (2011). Actin fusion proteins alter the dynamics of mechanically induced cytoskeleton rearrangement. *PLoS ONE* **6**, e22941.

Delgado-Álvarez, D. L., Callejas-Negrete, O. A., Gómez, N., Freitag, M., Roberson, R. W., Smith, L. G. and Mouriño-Pérez, R. R. (2010). Visualization of F-actin localization and dynamics with live cell markers in Neurospora crassa. *Fungal Genet. Biol.* **47**, 573-586.

D'Este, E., Kamin, D., Göttfert, F., El-Hady, A. and Hell, S. W. (2015). STED nanoscopy reveals the ubiquity of subcortical cytoskeleton periodicity in living neurons. *Cell Rep.* **10**, 1246-1251.

Dominguez, R. and Holmes, K. C. (2011). Actin structure and function. *Annu. Rev. Biophys.* **40**, 169-186.

Doyle, T. and Botstein, D. (1996). Movement of yeast cortical actin cytoskeleton visualized in vivo. *Proc. Natl. Acad. Sci. USA* **93**, 3886-3891.

Du, J., Fan, Y.-L., Chen, T.-L. and Feng, X.-Q. (2015). Lifeact and Utr230 induce distinct actin assemblies in cell nuclei. *Cytoskeleton* **72**, 570-575.

Duleh, S. N. and Welch, M. D. (2012). Regulation of integrin trafficking, cell adhesion, and cell migration by WASH and the Arp2/3 complex. *Cytoskeleton* **69**, 1047-1058.

Fiolka, R., Shao, L., Rego, E. H., Davidson, M. W. and Gustafsson, M. G. L. (2012). Time-lapse two-color 3D imaging of live cells with doubled resolution using structured illumination. *Proc. Natl. Acad. Sci. USA* **109**, 5311-5315.

Griffin, B. A., Adams, S. R. and Tsien, R. Y. (1998). Specific covalent labeling of recombinant protein molecules inside live cells. *Science* **281**, 269-272.

Grikscheit, K. Frank, T., Wang, Y. and Grosse, R. (2015). Junctional actin assembly is mediated by Formin-like 2 downstream of Rac1. *J. Cell Biol.* **209**, 367-376.

Grosse, R. and Vartiainen, M. K. (2013). To be or not to be assembled: progressing into nuclear actin filaments. *Nat. Rev. Mol. Cell Biol.* **14**, 693-697.

Gurniak, C. B. and Witke, W. (2007). HuGE, a novel GFP-actin-expressing mouse line for studying cytoskeletal dynamics. *Eur. J. Cell Biol.* **86**, 3-12.

Holmes, K. C., Popp, D., Gebhard, W. and Kabsch, W. (1990). Atomic model of the actin filament. *Nature* **347**, 44-49.

Holubcová, Z., Howard, G. and Schuh, M. (2013). Vesicles modulate an actin network for asymmetric spindle positioning. *Nat. Cell Biol.* **15**, 937-947.

Kellogg, D. R., Mitchison, T. J. and Alberts, B. M. (1988). Behaviour of microtubules and actin filaments in living Drosophila embryos. *Development* **103**, 675-686.

Keppler, A., Pick, H., Arrivoli, C., Vogel, H. and Johnsson, K. (2004). Labeling of fusion proteins with synthetic fluorophores in live cells. *Proc. Natl. Acad. Sci. USA* **101**, 9955-9959.

Kikkawa, M., Ishikawa, T., Nakata, T., Wakabayashi, T. and Hirokawa, N. (1994). Direct visualization of the microtubule lattice seam both in vitro and in vivo. *J. Cell Biol.* **127**, 1965-1971.

Koestler, S. A., Rottner, K., Lai, F., Block, J., Vinzenz, M. and Small, J. V. (2009). F- and G-actin concentrations in lamellipodia of moving cells. *PLoS ONE* **4**, e4810.

Kristó, I., Bajusz, I., Bajusz, C., Borkúti, P. and Vilmos, P. (2016). Actin, actin-binding proteins, and actin-related proteins in the nucleus. *Histochem. Cell Biol.* **145**, 373-388.

Langhorst, M. F., Genisyuerek, S. and Stuermer, C. A. O. (2006). Accumulation of FlAsH/Lumio Green in active mitochondria can be reversed by beta-mercaptoethanol for specific staining of tetracysteine-tagged proteins. *Histochem. Cell Biol.* **125**, 743-747.

Lazarides, E. and Weber, K. (1974). Actin antibody: the specific visualization of actin filaments in non-muscle cells. *Proc. Natl. Acad. Sci. USA* **71**, 2268-2272.

Lemieux, M. G., Janzen, D., Hwang, R., Roldan, J., Jarchum, I. and Knecht, D. A. (2014). Visualization of the actin cytoskeleton: different F-actin-binding probes tell different stories. *Cytoskeleton* **71**, 157-169.

Lin, A. Y., Prochniewicz, E., James, Z. M., Svensson, B. and Thomas, D. D. (2011). Large-scale opening of utrophin's tandem calponin homology (CH) domains upon actin binding by an induced-fit mechanism. *Proc. Natl. Acad. Sci. USA* **108**, 12729-12733.

Louvet, E. and Percipalle, P. (2008). Transcriptional control of gene expression by actin and myosin. *Int. Rev. Cell Mol. Biol.* **272**, 107-147.

Lukinavičius, G., Umezawa, K., Olivier, N., Honigmann, A., Yang, G., Plass, T., Mueller, V., Reymond, L., Corrêa, I. R., Jr, Luo, Z.-G. et al. (2013). A near-infrared fluorophore for live-cell super-resolution microscopy of cellular proteins. *Nat. chem.* **5**, 132-139.

Martin, B. R., Giepmans, B. N. G., Adams, S. R. and Tsien, R. Y. (2005). Mammalian cell-based optimization of the biarsenical-binding tetracysteine motif for improved fluorescence and affinity. *Nat. Biotechnol.* **23**, 1308-1314.

McGough, A., Pope, B., Chiu, W. and Weeds, A. (1997). Cofilin changes the twist of F-actin: implications for actin filament dynamics and cellular function. *J. Cell Biol.* **138**, 771-781.

Miralles, F., Posern, G., Zaromytidou, A.-I. and Treisman, R. (2003). Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* **113**, 329-342.

Mizoguchi, T., Kawakami, K. and Itoh, M. (2016). Zebrafish lines expressing UAS-driven red probes for monitoring cytoskeletal dynamics. *Genesis* **54**, 483-489.

Mounier, N., Perriard, J. C., Gabbiani, G. and Chaponnier, C. (1997). Transfected muscle and non-muscle actins are differentially sorted by cultured smooth muscle and non-muscle cells. *J. Cell Sci.* **110**, 839-846.

Munsie, L. N., Caron, N., Desmond, C. R. and Truant, R. (2009). Lifeact cannot visualize some forms of stress-induced twisted F-actin. *Nat. Methods* **6**, 317.

Neujahr, R., Heizer, C., Albrecht, R., Ecke, M., Schwartz, J.-M., Weber, I. and Gerisch, G. (1997). Three-dimensional patterns and redistribution of myosin II and actin in mitotic Dictyostelium cells. *J. Cell Biol.* **139**, 1793-1804.

Panza, P., Maier, J., Schmees, C., Rothbauer, U. and Sollner, C. (2015). Live imaging of endogenous protein dynamics in zebrafish using chromobodies. *Development* **142**, 1879-1884.

Perrin, B. J. and Ervasti, J. M. (2010). The actin gene family: function follows isoform. *Cytoskeleton* **67**, 630-634.

Phng, L.-K., Stanchi, F. and Gerhardt, H. (2013). Filopodia are dispensable for endothelial tip cell guidance. *Development* **140**, 4031-4040.

Plamont, M.-A., Billon-Denis, E., Maurin, S., Gauron, C., Pimenta, F. M., Specht, C. G., Shi, J., Quérard, J., Pan, B., Rossignol, J. et al. (2016). Small fluorescence-activating and absorption-shifting tag for tunable protein imaging in vivo. *Proc. Natl. Acad. Sci. USA* **113**, 497-502.

Plessner, M. and Grosse, R. (2015). Extracellular signaling cues for nuclear actin polymerization. *Eur. J. Cell Biol.* **94**, 359-362.

Plessner, M., Melak, M., Chinchilla, P., Baarlink, C. and Grosse, R. (2015). Nuclear F-actin formation and reorganization upon cell spreading. *J. Biol. Chem.* **290**, 11209-11216.

Riedl, J., Crevenna, A. H., Kessenbrock, K., Yu, J. H., Neukirchen, D., Bista, M., Bradke, F., Jenne, D., Holak, T. A., Werb, Z. et al. (2008). Lifeact: a versatile marker to visualize F-actin. *Nat. Methods* **5**, 605-607.

Riedl, J., Flynn, K. C., Raducanu, A., Gärtner, F., Beck, G., Bösl, M., Bradke, F., Massberg, S., Aszodi, A., Sixt, M. et al. (2010). Lifeact mice for studying F-actin dynamics. *Nat. Methods* **7**, 168-169.

Rocchetti, A., Hawes, C. and Kriechbaumer, V. (2014). Fluorescent labelling of the actin cytoskeleton in plants using a cameloid antibody. *Plant Methods* **10**, 12.

Rullo, J., Becker, H., Hyduk, S. J., Wong, J. C., Digby, G., Arora, P. D., Cano, A. P., Hartwig, J., McCulloch, C. A. and Cybulsky, M. I. (2012). Actin polymerization stabilizes $\alpha 4\beta 1$ integrin anchors that mediate monocyte adhesion. *J. Cell Biol.* **197**, 115-129.

Sanders, T. A., Llagostera, E. and Barna, M. (2013). Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning. *Nature* **497**, 628-632.

Schachtner, H., Li, A., Stevenson, D., Calaminus, S. D. J., Thomas, S. G., Watson, S. P., Sixt, M., Wedlich-Soldner, R., Strathdee, D. and Machesky,

L. M. (2012). Tissue inducible Lifeact expression allows visualization of actin dynamics in vivo and ex vivo. *Eur. J. Cell Biol.* **91**, 923-929.
Schell, M. J., Erneux, C. and Irvine, R. F. (2001). Inositol 1,4,5-Trisphosphate 3-Kinase A Associates with F-actin and Dendritic Spines via Its N terminus. *J. Biol. Chem.* **276**, 37537-37546.
Schuh, M. (2011). An actin-dependent mechanism for long-range vesicle transport. *Nat. Cell Biol.* **13**, 1431-1436.
Sherwin, T. and Gull, K. (1989). Visualization of detyrosination along single microtubules reveals novel mechanisms of assembly during cytoskeletal duplication in trypanosomes. *Cell* **57**, 211-221.
Sliogeryte, K., Thorpe, S. D., Wang, Z., Thompson, C. L., Gavara, N. and Knight, M. M. (2016). Differential effects of LifeAct-GFP and actin-GFP on cell mechanics assessed using micropipette aspiration. *J. Biomech.* **49**, 310-317.
Spracklen, A. J., Fagan, T. N., Lovander, K. E. and Tootle, T. L. (2014). The pros and cons of common actin labeling tools for visualizing actin dynamics during Drosophila oogenesis. *Dev. Biol.* **393**, 209-226.
Suarez, C., Carroll, R. T., Burke, T. A., Christensen, J. R., Bestul, A. J., Sees, J. A., James, M. L., Sirotkin, V. and Kovar, D. R. (2015). Profilin regulates F-actin network homeostasis by favoring formin over Arp2/3 complex. *Dev. Cell* **32**, 43-53.
Vandekerckhove, J., Deboben, A., Nassal, M. and Wieland, T. (1985). The phalloidin binding site of F-actin. *EMBO J.* **4**, 2815-2818.
Van Der Honing, H. S., Van Bezouwen, L. S., Emons, A. M. C. and Ketelaar, T. (2011). High expression of Lifeact in Arabidopsis thaliana reduces dynamic reorganization of actin filaments but does not affect plant development. *Cytoskeleton* **68**, 578-587.
Vavylonis, D., Kovar, D. R., O'shaughnessy, B. and Pollard, T. D. (2006). Model of formin-associated actin filament elongation. *Mol. Cell* **21**, 455-466.
Verkhusha, V. V., Tsukita, S. and Oda, H. (1999). Actin dynamics in lamellipodia of migrating border cells in the *Drosophila* ovary revealed by a GFP-actin fusion protein. *FEBS Lett.* **445**, 395-401.
Vidali, L., Rounds, C. M., Hepler, P. K. and Bezanilla, M. (2009). Lifeact-mEGFP reveals a dynamic apical F-actin network in tip growing plant cells. *PLoS ONE* **4**, e5744.
Visegrády, B., Lőrinczy, D., Hild, G., Somogyi, B. and Nyitrai, M. (2004). The effect of phalloidin and jasplakinolide on the flexibility and thermal stability of actin filaments. *FEBS Lett.* **565**, 163-166.
Wade, R. H. (2007). Microtubules: an overview. *Methods Mol. Med.* **137**, 1-16.
Wehland, J., Osborn, M. and Weber, K. (1977). Phalloidin-induced actin polymerization in the cytoplasm of cultured cells interferes with cell locomotion and growth. *Proc. Natl. Acad. Sci. USA* **74**, 5613-5617.
Westphal, M., Jungbluth, A., Heidecker, M., Mühlbauer, B., Heizer, C., Schwartz, J.-M., Marriott, G. and Gerisch, G. (1997). Microfilament dynamics during cell movement and chemotaxis monitored using a GFP–actin fusion protein. *Curr. Biol.* **7**, 176-183.
Winder, S. J., Hemmings, L., Bolton, S. J., Maciver, S. K., Tinsley, J. M., Davies, K. E., Critchley, D. R. and Kendrick-Jones, J. (1995). Calmodulin regulation of utrophin actin binding. *Biochem. Soc. Trans.* **23**, 397S.
Wu, J.-Q. and Pollard, T. D. (2005). Counting cytokinesis proteins globally and locally in fission yeast. *Science* **310**, 310-314.
Wulf, E., Deboben, A., Bautz, F. A., Faulstich, H. and Wieland, T. (1979). Fluorescent phallotoxin, a tool for the visualization of cellular actin. *Proc. Natl. Acad. Sci. USA* **76**, 4498-4502.