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Review

Are non-muscle actin isoforms functionally equivalent?

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Summary. Actin is highly conserved and it is the most widespread protein in eukaryotic cells. One of the most important features of actin, which allows it to have many different functions, is its ability to polymerize and interact with many other proteins. Actins are the major constituent of the actin cytoskeleton, which is an important system that is involved in various aspects of cell function, including cell motility, structure, integrity, regulation of signal transduction and transcription. Six mammal actin isoforms are highly conserved and share common functions. Two of them, β and γ non-muscle actin isoforms, which differ only by four amino acids located at the N-terminus of the polypeptide chain, are required for survival and proper cell functioning. We also summarized data about actbl2, which is suggested to be a newly discovered isoactin. Here, we review the current knowledge about tissue-specific expression of the non-muscle actin isoforms and possible functional differences between them. We also discuss molecular tools, which in recent years have allowed for a better understanding of the role of these proteins in cell functioning.

Key words: Non-muscle actin isoforms, β and γ actin isoformm, actbl2, Cell migration

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Introduction

Actin is the most abundant protein in eukaryotic cells. Its presence has been observed in the cytoplasm and nucleus, where it maintains the nuclear structure and participates in transcription and signal transduction processes (McDonald et al., 2006; Hofmann, 2009; Virtanen and Vartiainen, 2017). The main feature of actin is its ability to polymerize. The dynamic equilibrium between monomeric and polymerized actin continuously ensures that the actin cytoskeleton is able to fulfill its various roles in cell functioning. Among these roles are cell motility, contractile ring formation during cytokinesis, maintenance of cell shape, signal transduction, cell adhesion, transcription, and muscle contraction (Perrin and Ervasti, 2010; Ampe and Van Troys, 2016).

For years, there were known to be six highly conserved actin isoforms in vertebrates: four muscle and two non-muscle isoforms. They have been classified by both isoelectric point and primary tissue or cellular localization. The actin protein family comprises α skeletal actin, which is present in skeletal muscles, α cardiac actin, α and γ smooth muscle isoactins, and β and γ non-muscle (Vandekerckhove and Weber, 1978). Muscle actins are tissue specific, whereas β and γ non-muscle actins, which are encoded by *ACTB* and *ACTG1* genes respectively, are ubiquitously present in almost all cell types and are essential for cell survival (Harborth et al., 2001). During the last decade, another actin isoform—actbl2 (actin, beta like 2)—was observed at the protein level (Danielsen et al., 2011; Kim et al., 2011; Lopitz-Otsoa et al., 2012; Leng et al., 2014; Bober et al., 2016; Mazur et al., 2016; Ghazanfar et al., 2017).

Actin isoforms are products of separate genes, although there is a high homology among their nucleotide sequences resulting in a similar protein primary structure. The differences between actin isoforms occur especially in the most variable N-terminal region of the actin molecule (Sheterline et al., 1995; Khaitlina, 2001) (Fig. 1), and they affect the isoelectric point value, which varies from 5.2 to 5.7 (Nowak and Malicka-Błaszkiewicz, 1999). For example, non-muscle actins are slightly more alkaline than muscle actins (Vandekerckhove and Weber, 1978). Actin isoforms mirror tissue, but not species, specificity, which means that more differences in amino acid composition can be observed in actins originating from different tissues of the same species compared with actins originating from the same tissue of evolutionarily distant organisms (Sheterline et al., 1995).

β and γ non-muscle actin isoforms differ only by four amino acids located at positions 2, 3, 4, and 10 (Fig. 1). β actin contains Asp-Asp-Asp at the N-terminus and Val at position 10 of the polypeptide chain, whereas γ actin possesses the N-terminal tripeptide Glu-Glu-Glu and Ile at position 10 (Vandekerckhove and Weber, 1978). In addition to differences in amino acid sequences, these isoforms have different isoelectric points; β actin has a lower isoelectric point than γ actin (Bergeron et al., 2010).

Characteristics of non-muscle actins

In cells, actin is present in two forms: as a free monomer called globular actin (G-actin) or as a linear polymer called a microfilament (filamentous actin; F-actin), both of which co-exist in a dynamic equilibrium and are essential for important cellular functions such as motility and contraction of cells during cell division. One of the most important features of actin is its ability to polymerize and interact with many other proteins, which allows actin to have many different functions.

The actin molecule consists of two structural domains known as the large and the small domains, which are separated by the cleft where ADP or ATP binds (Kabsch et al., 1990). Each actin domain can be further subdivided into two subdomains. The small domain includes the subdomain I, containing both the N- and C-terminus of the molecule (comprising of residues 1-32, 70-144 and 338-372), and the subdomain II (residues 33-69). The larger domain is also divided into two subdomains, i.e. III (residues 145-180 and 270-337) and IV (residues 181-269) (Kabsch et al., 1990; Rould et al., 2006). Some authors call the subdomains Ia, Ib, IIa, and IIb, respectively. The actin monomer has several divalent cation-binding sites (especially Ca^{2+} and Mg^{2+}), which together with a nucleotide are responsible for maintaining the native conformation of actin (Sheterline et al., 1995). Under strict *in vitro* conditions, i.e. in the presence of ATP; at a specified concentration of Mg^{2+}, Ca^{2+}, and K^{+} ions; and at a critical concentration of monomer, the actin polymerization process occurs spontaneously and is accompanied by ATP hydrolysis. ADP remains bound within the actin filament, whereas phosphate residue is released. Actin filaments exhibit polarity; they have a quickly growing (+ or barbed) end with a high affinity for actin monomers, and a slow-growing end (- or pointed), with a low affinity for actin monomers. The exposed areas of subdomains I and III of the actin monomer are referred to the "barbed" ends, while the exposed areas of domains II and IV are termed the "pointed" ends. Under physiological conditions in the cells, the number of monomers binding to the (+) end is in equilibrium with a corresponding number of actin monomers dissociating from the (-) end. Actin monomers associate with the (+) end as polymerization progresses, and the monomers move along the filament, until they dissociate from the (-) end (Sheterline et al., 1995; Pollard and Borisy, 2003).

In recent years, a study conducted on non-muscle β and γ actins obtained by Baculovirus vector-mediated gene expression in insect cells indicated that these isoforms differ from each other in terms of the dynamics of the polymerization process (Bergeron et al., 2010). When calcium ions are associated with actin, ATP exchange within the β actin monomers occurred twice as fast as for γ actin isoform monomers. In addition, β actin was characterized as having higher polymerization dynamics (Bergeron et al., 2010). Authors suggest that one or more of the four amino acids that are different between the isoforms may affect the biological activity of these proteins causing the difference in the organization of the ATP binding pocket. Mg^{2+} ions are present in the cytoplasm in much higher concentration than Ca^{2+} ions at physiological conditions, and actin

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                          1                   40
α smooth muscle actin     MCEEEDSTAL VCDNGSGLCK AGFAGDDAPR AVFPSIVGRP
α cardiac actin           MCDDEETTAL VCDNGSGLVK AGFAGDDAPR AVFPSIVGRP
α skeletal muscle actin   MCDEDETTAL VCDNGSGLVK AGFAGDDAPR AVFPSIVGRP
β non-muscle actin        --MDDDIAAL VVDNGSGMCK AGFAGDDAPR AVFPSIVGRP
β like 2 actin            -MTDNELSAL VVDNGSGMCK AGFGGDDAPR AVFPSMIGRP
γ non-muscle actin        --MEEEIAAL VIDNGSGMCK AGFAGDDAPR AVFPSIVGRP
γ smooth muscle actin     MC-EEETTAL VCDNGSGLCK AGFAGDDAPR AVFPSIVGRP
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Fig. 1. Comparison of N-terminal sequences of actin isoforms. Bold blue sequences represent conservative amino acids in all isoforms. Amino acids residues, which differ between isoforms, are in black. Alignment was performed using a multi sequence alignment tool, Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) (Goujon et al., 2010).

associating with the Mg^{2+} might be more common. Under these conditions, differences in the polymerization of both isoforms are still present, but they are less significant. However, under some physiological and pathological conditions, the calcium ion concentration may change in cells. For example, the tumorigenesis process is often accompanied by a change in the intracellular concentration of calcium ions, which can cause a larger-than-normal cell diversification in polymerization dynamics of both non-muscle isoforms. It was also demonstrated that both isoforms may polymerize together to form a filament with intermediate properties between those composed exclusively of β or γ actin. It is possible that differential polymerization rates can create two types of filaments in cells, which have diverse dynamic properties (Bergeron et al., 2010; Perrin and Ervasti, 2010).

Localization and function of non-muscle actins

It is unclear why there are two such similar non-muscle isoforms of actin, whether they have different functions, and where they are located in the cell. Another important question is whether altered expression of non-muscle actin isoforms is related to the occurrence of certain pathological conditions in the body. The differences in polymerization of non-muscle actins (Bergeron et al., 2010) and in their interaction with proteins regulating this process (Pollard, 2016) suggest a hypothesis that they may fulfill different functions in cells. There is still a growing number of reports discussed below about different levels and subcellular distribution of β and γ actin in cells.

The ratio of the non-muscle actin isoform levels is not a constant value and it strictly depends on the cell type (Vandekerckhove and Weber, 1981; Otey et al., 1987; Sheterline et al., 1995). The ratio of β-to-γ isoforms in various rat tissues is in the range of 1:1 in the testis, 2.5:1 in the liver, and 6:1 in the aorta (Vandekerckhove and Weber, 1981; Otey et al., 1987). However, typically the ratio of isoforms β:γ is 2:1 (Khaitlina, 2001; Bergeron et al., 2010). However, there are exceptions to this rule. In the auditory hair cells, the ratio is reversed (i.e. γ actin is on a higher level than β actin (Höfer et al., 1997)), whereas mammalian erythrocytes contain only β actin (Pinder and Gratzer, 1983). Additionally, in human tissues, as shown by Ampe and van Troys (2016), the ratio between β and γ actin mRNA level is also variable. In some tissues or cells, β actin dominates (e.g. granulocytes), while in others, γ actin is expressed at higher levels (e.g. adult stem cells or testis). However, in most cell types or tissues, the level of both non-muscle actin isoforms is similar.

During embryonic development in rats, the non-muscle actin isoforms dominate independent of tissue type, (McHugh et al., 1991). It was also suggested that, in the overall actin pool, non-muscle actin mRNA is present at the early stages of mouse oogenesis and in embryos, and this mRNA encoding the non-muscle actin isoforms is the most common (Bachvarova et al., 1989). β and γ actins are also the only actin isoforms synthesized during meiosis in oocytes (Brockmann et al., 2011). During smooth muscle cell differentiation, the level of β actin is reduced and α smooth muscle actin becomes the predominant isoform. Myogenesis of skeletal muscles is also characterized by depletion of non-muscle forms of actin, with concomitant increasing levels of muscle-specific isoforms (Lloyd et al., 2004). β actin expression is significantly reduced in contrast to the γ isoform, which is located in costameres and the plasma membrane of mature skeletal muscle (Rybakova et al., 2000; Lloyd et al., 2004).

Changes in the level of actin isoform gene expression often accompany pathological processes. The number of diseases related to the qualitative and quantitative changes of actins (like mutations, changes in gene expression or cellular level, polymerization status) is still growing (Nunoi et al., 1999; Procaccio et al., 2006; Rivière et al., 2013; Di Donato et al., 2014; Hundt et al., 2014; Miyagawa et al., 2015).

Increased levels of non-muscle actin isoforms accompany many types of tumors, such as chemically induced skin cancer, liver cancer, lymphoma, and breast cancer (discussed by Nowak and Malicka-Błaszkiewicz, 1999). The level of β actin was often observed to be increased in highly-invasive cancer cells (Le et al., 1998; Nowak et al., 2005). A significant increase in its concentration was noticed in selected, invasive colon carcinoma lines (Nowak et al., 2005), and the Madin-Darby canine kidney (MDCK) cell line transformed with Moloney sarcoma virus (MSV) (Le et al., 1998), as well as in melanoma T1C1 cells (Goidin et al., 2001). The level of β actin gene expression is also increased in a very invasive colon carcinoma cell line selected by paxlitaxel treatment compared with the parental cell line (Dowling et al., 2007). Conversely, mutation in the gene encoding γ actin results in inhibition or reduction of its synthesis, which causes resistance of acute lymphoblastic leukemia cells to agents targeting microtubules (Verrills et al., 2006). Lymphocytes express β actin in remarkable excess over the γ actin, whereas their leukemic counterparts synthetize both isoforms in equal proportions (Nagata and Ichikawa, 1984). Dugina and co-workers also observed a significant decrease of β actin expression in non-small cell lung cancer compared with non-malignant tissue. Conversely, γ actin expression was doubled in carcinoma compared with normal tissues. Similar results were obtained for colon cancer, i.e. five times lower intensity of β actin and about double enhancement of γ actin staining in neoplastic vs. normal cells (Dugina et al., 2015). Additionally, it was shown that during epithelial-mesenchymal transition in cervical carcinoma cells, reorganization of β actin structures and downregulation of this isoform expression occurs (Shagieva et al., 2012).

Disturbances in actin isoform expression are not

only associated with cancer cells. Single point mutations within the β actin gene (Glu364Lys) result in formation of dysfunctional neutrophils with a weaker chemotactic response and reduced ability to form peroxides (Nunoi et al., 1999). Additionally, Hundt and colleagues showed that actin gene mutations can lead to changes in actin conformation. During ADP for ATP exchange, actin has to undergo transitions between open and closed states that involve twisting of its two major domains. Any impairment in the conformational flexibility associated with these transitions is predicted to affect nucleotide exchange and polymerization behavior. Hundt and co-workers showed that replacement of a glutamic acid at position 364 in the polypeptide chain by a lysine residue can trigger events leading to the preferred formation of the closed β actin state. This mutation affects interdomain mobility and perturbs exchange of ADP-actin to ATP-actin monomers, which may be a basis for the formation of disease phenotypes in patients, which is manifested as neutrophil dysfunction (Hundt et al., 2014). The Arg183Trp mutation in the same gene causes resistance of filaments to the depolymeryzation by cofilin. This leads to malformation during fetal development, deafness, and dystonia (Procaccio et al., 2006). Biochemical studies indicated that arginine replacement by a tryptophan residue at position 183 establishes an unusual stacking interaction with Tyr69 that perturbs nucleotide release from actin monomers and polymerization behavior by inducing a closed state conformation (Hundt et al., 2014). Mutations of the *ACTG1* (γ actin) gene are also often responsible for hearing loss (van Wijk et al., 2003; Morín et al., 2009; Miyagawa et al., 2015). Among them is Thr278Ile mutation in non-muscle γ actin gene. This mutation weakens all the filaments, thereby disturbing the process of stereocilia renewal (van Wijk et al., 2003). The stereocilium, which is structured by parallel actin filaments, is composed of both actin isoforms, and is the responsive organelle to mechanical stimuli such as sound, gravity, and head movements. Additionally, Miyagawa et al. (2015) and Morín et al. (2009) identified four other *ACTG1* mutations in this gene that lead to progressive hearing loss. Moreover, mutations in β and γ actin genes have been recently reported to cause Baraitser-Winter syndrome, a rare but well-defined developmental disorder recognized by the combination of congenital ptosis, high-arched eyebrows, hypertelorism, ocular colobomata and a brain malformation consisting of anterior predominant lissencephaly (Rivière et al., 2013; Di Donato et al., 2014; Rubenstein and Wen, 2014).

The level of γ actin is also changed in the case of Duchenne muscular dystrophy. In this disease a mutation in a gene encoding dystrophin functional protein inhibits its production. In skeletal muscles lacking dystrophin, a significant increase in the γ actin level was reported, which may at least partially compensate for the lack of dystrophin (Prins et al., 2008) (Table 1).

Many older studies suggested that β actin is present in the migrating cells in the submembranous area and at the leading edge of the cell, where it forms protrusions. This isoform was shown to play a role in migration and wound healing. In contrast, γ actin was shown to locate within stress fibers, where it is responsible for maintaining the cell shape and resisting mechanical stress (Hoock et al., 1991; Khaitlina, 2001). However, the presence of γ actin at the leading edge was not excluded, but rather its detection was difficult, because of a lack of sufficiently specific antibodies. Thus, this isoform was indirectly detected and localized in the cell areas that were rich in filamentous actin and but that showed no β actin. However, in recent years, new antibodies were developed that are selectively directed against specific actin isoforms; it was shown that β actin is also present in the stress fibers and γ actin occurs at the leading edge of the migrating cells. Studies have suggested that in fibroblasts, β actin is preferentially localized in stress fibers, circular bundles, and at cell-

Table 1. Effects of mutations in genes encoding β and γ non-muscle actin isoforms.

Type of mutation	Effect of mutation	Reference
Neutrophils, a point mutation in β actin gene (Glu364Lys)	Reduced chemotactic cell response and reduced peroxide forming ability; resulting in mental disability, sensitivity to light, prone to infections	Nunoi et al., 1999; Hundt et al., 2014
Point mutation in β actin gene (Arg183Trp)	Formation of stable filaments, lack of depolymerization; resulting in distortion during fetal development, hearing impairment, dystonia	Procaccio et al., 2006; Hundt et al., 2014
Point mutation in γ actin gene (Thr278Ile)	Weakness of filaments, disorders in stereocilia renewal; resulting in hearing loss	van Wijk et al., 2003
Mutations in the γ actin gene, which reduce the level of γ actin (Val103Leu, Asp187His, Thr162Met, Pro98Leu)	Acute lymphoblastic leukemia is less sensitive to agents targeting microtubules	Verrills et al., 2006
Point mutation in γ actin gene, DFNA20/26 (Lys118Asn; Glu241Lys; Gly48Arg; Leu229Val)	Hearing impairment connected to autosomal dominant sensorineural hearing loss	Morín et al., 2009; Miyagawa et al., 2015
Point mutations in β actin gene (Arg196His) and γ actin gene (Ser155Phe)	Baraitser-Winter syndrome, developmental disorder recognized by the combination of congenital ptosis, high-arched eyebrows, hypertelorism, ocular colobomata and a brain malformation consisting of anterior predominant lissencephaly	Rivière et al., 2013; Di Donato et al., 2014

cell contacts, whereas γ actin is mainly organized as a meshwork in cortical and lamellipodia structures (Dugina et al., 2009). Differences in the relative localization of actin isoforms compared with previous data may be because the availability of protein epitopes strongly depends on cell fixation and permeabilization conditions.

Non-muscle actin isoforms are also differentially localized in different cell types. Baranwal and co-workers proposed that intracellular actin levels are a key regulator of epithelial junctions and that both non-muscle actin isoforms are essential for various aspects of apical junctional complex remodeling. β actin seems to be essential for maintenance of adherens junctions and it is required for normal epithelial tight junction structure. The γ isoform was shown to be important for maintenance of tight junction integrity, but its role in maintenance of adherens junctions was not determined (Baranwal et al., 2012). Moreover, Cruz et al. (2015) showed that spatially localized β actin translation takes part in cytoskeletal remodeling and consequently E-cadherin clustering at cell-cell contact sites, and therefore positively regulates adherens junction assembly. It was also demonstrated that β and γ actins play different roles in other cellular processes such as oocyte meiosis (Brockmann et al., 2011) or epithelial-mesenchymal transition in cervical carcinoma cells (Shagieva et al., 2012). During this process, reorganization of β actin structures and downregulation of its expression occurs. Both isoforms were also observed in cell nuclei. Immunolocalization studies demonstrated that both actins colocalize with RNA polymerase II and hnRNP U, and the β actin level has a significantly higher nucleus-to-cytoplasm ratio than the γ actin level (Migocka-Patrzałek et al., 2015). These isoforms also demonstrated functional diversification in the gene transcription process (Zheng et al., 2009).

Some results from studies to determine the actin localization in stereocilia of inner ear hair cells were unclear. Using immunofluorescent techniques, it was demonstrated that γ actin is located at its periphery (Belyantseva et al., 2009). Studies using electron microscopy indicated that β actin was located at its periphery, whereas the γ isoform was present in the central part of stereocilia (Furness et al., 2005). Another electron microscopy study in mice showed that both β and γ actin isoforms colocalize throughout stereocilia during embryonic development. Immunohistochemical analysis indicated that there was 40% more γ actin than β actin. In contrast, β and γ actin in adult stereocilia were equally distributed (Andrade, 2015).

There are also reports about the localization of the non-muscle actin isoforms in cancer cells. However, depending on the cell line used in studies, different results were obtained. Le and co-workers showed that in MDCK cells transformed with MSV virus, β actin is located only at the edges of pseudopodia, while γ actin was additionally present inside the cell (Le et al., 1998). Our experimental results indicate that both non-muscle actin isoforms are equally present in mesenchymally migrating cells in the areas connected with cell movement, i.e. lamellipodia and invadopodia (Simiczyjew et al., 2014, 2015). Shum et al. (2011) showed that neuroblastoma cells contain stress fibers that are rich mainly in β actin, whereas γ actin is present in peripheral cell regions, near the lamellipodium. Their results were confirmed by Dugina and colleagues (2015) who postulated that β non-muscle actin acts as a tumor suppressor by inhibiting cell growth and invasion, while γ non-muscle actin increases the oncogenic potential of the cells via interaction with ERK1/2, p34-Arc, WAVE2, cofilin1, PP1, and other regulatory proteins.

The small differences between β and γ actin amino acid sequences that cause the determination of non-muscle actin isoform localization represents a great challenge and antibodies with high specificity are necessary for studies. However, even when good quality antibodies are used, the experimental results are largely affected by the experimental conditions (e.g. cell fixation method). Additionally, some actin-rich structures, such as costameres, central regions of stereocilia or stress fibers, are difficult to identify. During analysis, actin filaments present in stereocilia or stress fibers may remain unstained because of epitope masking or limited penetration of antibodies within these structures. In addition, the structure of costameres is unstable and can be easily damaged during fixation and visualization (Franke et al., 1996; Perrin and Ervasti, 2010).

One mechanism regulating localization of actins is present already at the posttranscriptional level. The relationship between localization of actin isoforms and localization of mRNA encoding the isoforms has been shown. Moreover, the correlation between cellular localization of β actin mRNA and the migration capacity of cells was demonstrated (Shestakova et al., 1999). In several types of cells, β actin mRNA is located near the leading edge, which contributes to the formation of migratory protrusions and affects the direction of cell movement. It has been shown that translocation of this mRNA from the leading edge to the center of the cell results in the loss of its polarity and directional migration abilities (Kislauskis et al., 1997; Condeelis and Singer, 2005). A short sequence called “zipcode”, which is present within its 3'UTR regions, are responsible for appropriate β actin mRNA localization (Condeelis and Singer, 2005). In the nucleus, zipcode binding protein 1 (ZBP1) binds to this sequence and next translocates β actin mRNA through nuclear pores into the cytoplasm, where, with help of motor proteins such as KIF11 (a microtubule motor), it is transported along microtubules to the areas where actin-rich protrusions are formed (lamellipodia, filopodia) (Oleynikov and Singer, 2003; Song et al., 2015). Interaction of β actin mRNA with ZBP1 protects it from formation of a translational complex. Locked mRNA is transported to an appropriate localization, where it is released after phosphorylation of ZBP1 by Src kinase. This modification causes dissociation of mRNA from the

ZBP1 protein, ribosome complex formation, and β actin synthesis at the leading edge of cells (Farina et al., 2003; Hüttelmaier et al., 2005; Khaitlina, 2007). The same mechanism regulates translocation of β actin mRNA in neuronal cells, where actin is involved in the neurons' response to signals from the environment (Ming, 2006). In addition, ZBP1 induction of molecular pathways leads to augmented actin polymerization, promotes formation of migratory protrusions, and increases the rate of cell migration (Stöhr and Hüttelmaier, 2012). However, localization of actin mRNA does not always determine its post-translational localization. For example in myoblasts, γ actin mRNA is localized in the perinuclear area and in the cytoplasm, whereas the synthetized protein is present in the stress fibers and submembranous regions (Hill and Gunning, 1993). For γ actin mRNA, the mechanism regulating its localization has not yet been described. Thus, localization of mRNA encoding actin isoforms is not the only mechanism that determines the subsequent protein's location.

It was also shown that another RNA-binding protein, HuR, stabilizes the β actin mRNA by associating with a uridine-rich element within its 3'UTR. This protein plays an important role in mRNA stabilization, but not in the nuclear/cytoplasmic distribution of the β actin mRNA. Depletion of this protein in HeLa cells altered the β actin organization, and the consequent effects were correlated with loss of the actin stress fiber network in processes such as cell adhesion, migration, and invasion (Dormoy-Raclet et al., 2007).

Additionally, Ghosh and colleagues showed that the non-muscle β actin gene, ACTB, may generate two alternative transcripts that terminate at tandem polyA sites. β actin mRNA, except for a standard polyA signal, also has a non-canonical site upstream, just after the translation stop site (Ghosh et al., 2008). This enables cells to produce two transcripts with distinct properties. Ghosh et al.'s analysis indicated that longer transcripts are regulated in a tissue-specific manner, whereas the proximal polyA site is used for constitutive expression. The shorter variant is highly expressed, while the longer mRNA fragment has a significantly reduced expression level, but it is more stable, and thus it is translated much more efficiently. Translation of a longer transcript is under miRNA (miR-34) regulation. The purpose of the existence of two β actin mRNA variants is that by having unique expression properties, tissue-specific regulation may be achievable. This hypothesis is strengthened by the fact that the longer β actin mRNA variant, which is expressed at a low level in most tissues, exists at an increased level in the brain and it is present in drastically higher amounts in embryonic and germinal tissue (Ghosh et al., 2008; Artman et al., 2014).

There also exists a mechanism regulating the γ actin mRNA level. Drummond and co-workers identified a novel γ actin transcript that includes a previously unidentified exon. Inclusion of this exon introduces an in-frame termination codon. It has been hypothesized that this alternatively spliced mRNA reduces the γ actin level by targeting these transcripts for nonsense-mediated decay (Drummond and Friderici, 2013). The identified exon is predominantly expressed in skeletal muscle, cardiac muscle, and in the diaphragm. Drummond and Friderici (2013) suggest that this posttranscriptional regulation occurs in a process that was previously described as regulated unproductive splicing and translation (RUST). RUST occurs by alternative splicing to include a regulatory exon, which either contains or creates a premature termination codon (PTC) via frameshift. Introduction of a PTC results in subsequent degradation of the mRNA by nonsense-mediated decay (Lewis et al., 2003).

In recent years, it was demonstrated that non-muscle actin isoforms can be regulated at the transcriptional or translational level, and also by posttranslational modifications. Arginylation was described as a mechanism that regulates localization and function of non-muscle actin isoforms in cells. Both β and γ actin may undergo arginylation, but after this modification, γ actin becomes much less stable than β actin and is quickly ubiquitinated and degraded (Zhang et al., 2010). The process of arginylation is mediated by protein transferase 1 Arg-tRNA (Ate 1), which transfers an arginine residue at Asp, Glu, or Cys residue located in the N-terminus area of the polypeptide chain. An arginine residue on the N-terminal end of the β actin molecule alters the organization of the filaments. Arginylated β actin polymerizes in the form of a single filament, while non modified γ actin forms thick, parallel filament fibers (Karakozova et al., 2006). Thus, it is possible to maintain two separate forms of polymerized actin in a cell. Before arginylation, filaments composed of β actin are negatively charged because of the presence of aspartic acid and an N-terminal acetyl residue. The addition of arginine introduces an additional positive charge on the filament surface. Every fifth actin monomer is arginylated, and therefore, the formation of filaments from acetylated and arginylated forms lead to the appearance of filaments that are homogenously "coated" with positive charge. This prevents filaments from forming aggregates, as can be observed in negatively charged filaments (Karakozova et al., 2006). It is postulated that the leading edge of the cell may comprise a network of actin filaments that are formed by individual β actin filaments, while in the cell, thick γ actin fibers dominate in the center (Bulinski, 2006; Karakozova et al., 2006; Kashina, 2006; Terman and Kashina, 2013). Additionally, Pavlyk and co-workers showed that arginine deprivation modifies glioblastoma cell morphology, adhesion, migration, and invasiveness. These changes were associated with specific remodeling of the actin cytoskeleton organization caused by a decrease in β actin arginylation (Pavlyk et al., 2015).

Actin is also subjected to other posttranstational modifications such as acetylation, methylation, oxidation, phosphorylation, and ubiquitination (Terman and Kashina, 2013). It was also shown that nuclear actin

can be SUMOylated. In this process, small ubiquitin-related modifier (SUMO) binds to specific lysine residues on target proteins. SUMOylation of actin has been linked to transcription, regulation of the nuclear trafficking of actin and protein-protein interactions that are often related to nuclear functions (Hofmann et al., 2009). Both non-muscle actin isoforms are modified as described above, so that functional diversification between them cannot be observed in that field.

In addition to differences in non-muscle actin isoform level and localization, interaction with other proteins also varies. It was shown that β actin more strongly activates non-muscle myosin 2C1, while γ actin preferentially activates myosin 7A (Müller et al., 2013). Dugina and colleagues (2015) used co-immunoprecipitation experiments conducted on lung adenocarcinoma A549 cells and showed that Arp2/3 complex and cofilin 1 may preferentially interact with γ, rather than β, actin. However, Tzima and co-workers (2000) showed that annexin V may preferentially bind to γ actin and not to β actin in platelets. All these data are interesting, but as suggested by Ampe and Van Troys (2016), the β-to-γ actin ratio may determine results of such experiments and thus, these experiments should be conducted using purified proteins. This was confirmed by Lechuga and colleagues (2014), who showed that the transcription factor MRTFA interacts preferentially with γ non-muscle actin in A549 cells, but after silencing γ actin expression, β actin also formed complexes with MRTFA.

Molecular tools in study of non-muscle actins

For many years researchers investigated the role of non-muscle actin isoforms using molecular biology tools, in particular silencing or overexpression of genes encoding these isoforms (Schevzov et al., 1992; Peckham et al., 2001; Shmerling et al., 2005; Belyantseva et al., 2009; Bunnell and Ervasti, 2010; Tondeleir et al., 2012). However, the results remain controversial (Table 2), because these experiments were usually not very efficient (Schevzov et al., 1992; Choidas et al., 1998; Dugina et al., 2009; Shum et al., 2011). This may be because actin is a ubiquitously expressed protein that is necessary for main cellular functions, and its expression is strictly controlled. Treisman's group described serum response factor (SRF), which may regulate actin gene expression (Sotiropoulos et al., 1999; Posern and Treisman, 2006). SRF is an evolutionarily conserved nuclear transcription factor that mediates a rapid response to extracellular factors such as growth factors (Arsenian et al., 1998). In cells with overexpression of actin its monomeric form was demonstrated to decrease SRF levels, causing a significant reduction in expression of genes that are regulated by this factor including β and γ actin genes (Sotiropoulos et al., 1999; Posern et al., 2002).

Actin overexpression is usually obtained by expressing in cell plasmids containing cDNA that encodes particular isoactins that are tagged with a fluorescent protein. This enables visualization of this fusion protein within cells. β actin overexpression induced changes in cell morphology, formation of larger cellular protrusions and influenced cellular mobility, which was detected by doubling of migration speed compared with non-transfected cells. Changes in the level of actin binding proteins, such as cofilin and thymosin β, accompanied upregulation of β actin (Peckham et al., 2001). Transfection of murine myoblasts with a plasmid encoding β actin induced formation of the cell population with an increased cell

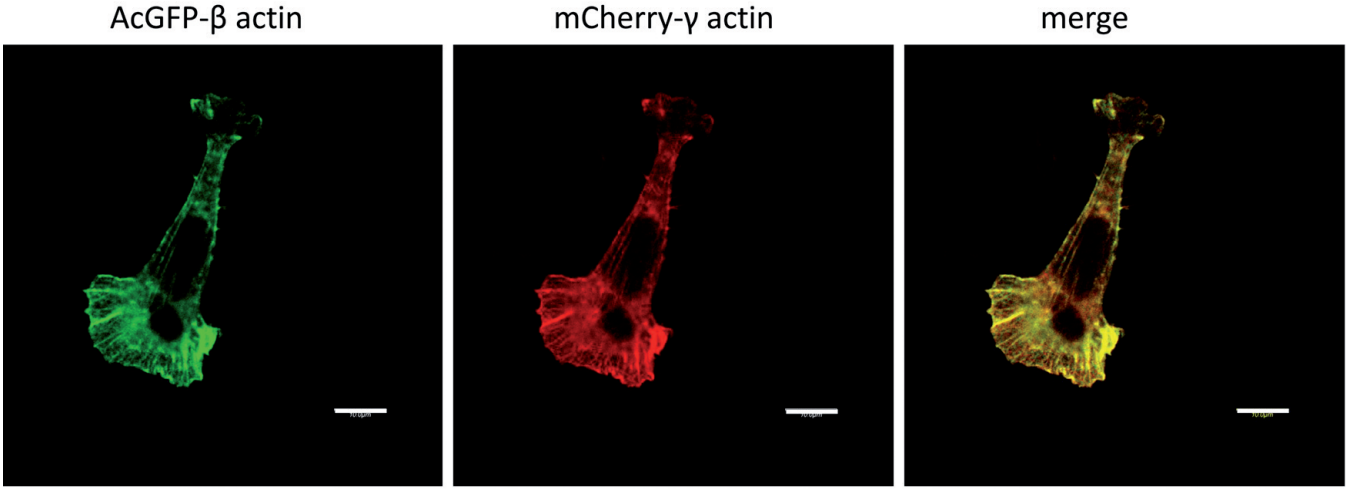

Fig. 2. Confocal images showing cells simultaneously overexpressing AcGFP-β actin and mCherry-γ actin. MDA-MB-231 cells were transfected with plasmids encoding pAcGFP-β actin or pmCherry-γ actin. Left picture: AcGFP-β actin; middle picture: mCherry-γ actin. Merged images are shown in the right panel. Scale bar: 10 μm.

surface area, whereas in constructs encoding γ actin, cells with a reduced area were observed. Studies also showed that, unlike γ actin, when β actin was overexpressed, cells had an expanded, well organized cytoskeleton. Thus suggests that in myoblasts, the actin cytoskeleton network is regulated by the β-to-γ actin level ratio (Schevzov et al., 1992). Our results, obtained on mesenchymally migrating cancer cells overexpressing actin isoforms, showed that both actin isoforms are present in protrusive actin-rich structures, such as lamellipodia and are engaged in migration of these cells (Simiczyjew et al., 2014) (Fig. 2). We also indicated that both of them are equally involved in formation of active invadopodia (Simiczyjew et al., 2015). To trigger overexpression of both isoforms we prepared plasmids pAcGFP-C1 containing cDNAs of β and γ actins with their 3'UTRs. We decided to preserve 3'UTRs because it is known that these mRNA regions are important for proper localization of at least β actin in the cell (Ross et al., 1997; Kislauskis et al., 1997; Condeelis and Singer, 2005). Cells transfected with these constructs expressed human β or γ actin isoforms tagged at the N-terminus of the polypeptide chain with a green fluorescent protein from *Aequorea coerulescens*

Table 2. Effects of changes in β and γ actin expression level on cellular functions.

Cell type	Overexpression/ silencing/knockout	Observed changes and effect on cellular functions	Reference
Murine myoblasts	β actin overexpression	- Increased cell surface area - Expanded, well organized cytoskeleton	Schevzov et al., 1992
	γ actin overexpression	- Decreased cell surface area - Less organized actin filament network	
Murine myoblasts	β actin overexpression	- Changes in cell morphology: increased cellular protrusion area - Increased migration speed	Peckham et al., 2001
Mesenchymally migrating human cancer cells	β actin overexpression	-Increased migration and invasion capacity -Overexpressed actins localized at the submembranous region of the cell body, especially within lamellipodia and invadopodia	Simiczyjew et al., 2014, 2015
	γ actin overexpression	-Increased migration and invasion capacity -Overexpressed actins localized at the submembranous region of the cell body, especially within lamellipodia and invadopodia	
HaCaT keratinocytes, lung cancer cells A549 and colon cancer HCT116 cells	β actin overexpression	-Decreases proliferation and invasiveness of examined cells	Dugina et al., 2015
	γ actin overexpression	-Increases proliferation and invasiveness of examined cells	
	β actin silencing	-Cells exhibited spread morphology, higher invasion capacity in cancer cells and lower in keratinocytes	
	γ actin silencing	-Cells showed contractile phenotype, lower invasion capacity in all examined cells	
Rat fibroblasts	β actin silencing	- Increased cell surface area - Reduced number of stress fibers - Bigger cell protrusions	Dugina et al., 2009
	γ actin silencing	- Shrunk phenotype - Formation of thick actin filaments - Decreased lamellipodia area - Decreased migration	
Human neuroblastoma cells	γ actin silencing	- Decreased migration speed - Cell polarity lost - Higher number of focal adhesions	Shum et al., 2011
Mouse/murine embryonal fibroblasts	β actin gene knockout	- Lethal at the embryonic stage E8.5 - Severe cell growth impairment - Decreased dynamics of cellular protrusions formation - Increased number of focal adhesions - Decreased migration speed	Bunnell et al., 2011
Murine embryonal fibroblasts	β actin gene knockout	- Changes in cell morphology: reduced protrusion length - Reduced migration ability	Tondeleir et al., 2012
Mouse	β actin gene knockout	- Very low expression level of transcription factor Gata2, which is necessary for early erythropoiesis and thus survival of the organism	Tondeleir et al., 2013
Mouse	γ actin gene knockout	- Increased mortality - Deafness	Belyantseva et al., 2009
Mouse/murine embryonal fibroblasts	γ actin gene knockout	- Most mice died within 48 hours after birth - Growth disorders - Limited survival - No changes in cellular migration	Bunnell and Ervasti, 2010
Human vascular endothelial cells	γ actin knockdown	- Significantly decreased cell motility and migration - No effect on cell adhesion to various substrates	Pasquier et al., 2015

(AcGFP). This fluorochrome is known to be present only as a monomer in a cell and does not form any aggregates as is often observed for GFP or EGFP (Jain et al., 2001; Gurskaya et al., 2003). To exclude disruption of the actin folding process by tagging AcGFP to the C-terminus (Brault et al., 1999; Rommelaere et al., 2004), we attached green fluorescent protein to actin's N-terminus. Dugina and co-workers (2015) postulate that overexpression of γ actin, which appears as a result of the transduction of HaCaT keratinocytes, lung cancer A549 cells, and colon cancer HCT116 cells with the lentiviral vector containing cDNA for this isoform, increases proliferation and invasiveness of these cells, while β actin overexpression exerts the opposite effect.

Researchers also tried to establish the role of non-muscle actin isoforms by silencing their gene expression, usually using siRNA or shRNA. Shum and colleagues used specific siRNA fragments and demonstrated that γ actin expression was reduced in neuroblastoma cells, leading to decreased cell migration speed, which was tested using the wound healing assay (Shum et al., 2011). Dugina et al. (2009) transfected cells using siRNA targeting β and γ actin sequences, and they also showed that when fibroblast β actin expression was reduced, cells showed increased cell surface area, formed many protrusions, and decreased stress fiber levels. However, in cells with silenced γ actin expression "shrunk" cell morphology was observed with thick actin filaments and reduced surface of lamellipodia-like structures. These data seem to be opposite to the results obtained when non-muscle actin isoforms are overexpressed in myoblasts, as described by Peckham et al. (2001). Latham et al. (2013) also showed that in endothelial hCMEC/D3 cells, γ actin is localized in the submembranous region, around the cell nucleus, and in the cell periphery, whereas β actin was mainly at the cell periphery. After tumor necrosis factor (TNF) stimulation, the two isoforms were asymmetrically distributed, with β actin stress fibers prominent at the basal surface and γ actin concentrated apically in a submembranous network. Differences in observed isoform distribution may be because cells with variations in their cytoskeleton organization were used in the described experiments. In addition, fibroblasts with a reduced expression of either β or γ actin showed changes in cellular motility compared with non-transfected control cells, suggesting that both non-muscle actin isoforms play a specific role in regulation of cell migration (Dugina et al., 2009). Recently, Dugina and co-workers (2015) conducted experiments using keratinocytes, lung cancer, and colon cancer cells, and they showed that silencing of both non-muscle actin isoforms using shRNA led to inhibition of cell proliferation. Cells with silenced β actin exhibited spread morphology, while cells with downregulated γ actin showed a "shrunk" phenotype. Studies also showed that lowered β actin level leads to higher invasion capacity, while decreased γ actin level reduced invasion capacity of the examined cancer cells. For keratinocytes, silencing of both isoforms inhibited cell invasion. Moreover, Pasquier et al. (2015) indicated that both non-muscle actin isoforms strongly colocalize in vascular endothelial cells, but with some degree of spatial preference. β actin was more enriched in radial stress fibers and membrane ruffling compared with γ actin, which was more uniformly spread across the entire microfilament meshwork. While β actin knockdown was not achievable in these cells without major cytotoxicity, γ actin knockdown significantly decreased their motility and migration, but had no effect on endothelial cell adhesion to various substrates. Po'uha and colleagues demonstrated that, by silencing γ actin expression, this isoform can modulate microtubule dynamics and is required to maintain centrosome integrity and regulate mitotic progression (Po'uha et al., 2013; Po'uha and Kavallaris, 2015). Additionally, Dugina et al. (2016) postulated that microtubule plus-end-tracking protein (end-binding 1 (EB1) interacts mainly with γ actin, and not β actin, in epithelial cells.

Because silencing using siRNAs or shRNAs only allows a partial reduction in the level of actin, some researchers chose to knockout these genes, which completely eliminates β or γ actin from the cell, and thus, we can obtain a better understanding of their roles. Knockout of β actin in mouse models was lethal during the embryonic or perinatal period (Shmerling et al., 2005; Bunnell et al., 2011). In myofibroblasts isolated in the early embryonic stage, it was shown that β actin knockout leads to higher expression of γ actin and α smooth muscle actin to compensate for the deficiency in total actin within the cell (Bunnell et al., 2011; Tondeleir et al., 2012). The effect of compensation after β actin knockout was not detected in CD4-positive T cells, but in both cases, depriving cells of this isoform caused impaired migration and appearance of more stress fibers and focal adhesions (Bunnell et al., 2011; Tondeleir et al., 2012). β actin gene knockout also resulted in upregulated expression of proteins engaged in actin remodeling, which contained LIM domains, EF hand structures, or calponin homology domains (Ampe et al., 2013). These may be connected with the observed stronger adhesion and contractility. Ampe's group showed that knockout of β actin in embryos leads to a low expression level of transcription factor Gata2, which is necessary for early erythropoiesis and thus survival of the organism (Tondeleir et al., 2013). Based on these results, they proposed that the reason for β actin devoid mouse embryos lethality can be not impairment of cells motility, but rather it impairs the role that β actin plays in the cell nucleus (Ampe and Van Troys, 2016). For γ actin knock out mice, only a small percentage survived until adulthood and this significant mortality was caused by developmental disorders and delays (Bunnell and Ervasti, 2010). Additionally, fibroblasts isolated from knockout mice had a limited amount of cell divisions, with apoptosis and necrosis being more frequently observed (Bunnell and Ervasti, 2010; Belyantseva et al.,

2009). However, compared with 100% lethality caused by the β actin knockout, we can conclude that these isoforms fulfill different functions in embryo development.

Care must be taken during analysis of data obtained from silencing of one of the actin isoforms, because as suggested by Ampe and Van Troys (2016), silencing of one isoform can induce an increase in the level of the other isoactin. This means that an observed effect may be a result of the reduced amount of the particular isoform or an elevated level of the other non-muscle actin isoform.

Actbl2, another actin isoform?

β actin-like protein 2 (Actbl2) is 92% identical to β actin. Actbl2 is suggested to be another actin isoform, because analysis of its amino acid sequence reveals the presence of an "actin conserved site" (IPR004001), according to the InterPro database, protein sequence analysis, and classification database (Hunter et al., 2012), which shows two actin signatures (1 and 2) that are published online in the PROSITE database (Sigrist et al., 2013; Mazur et al., 2016). For a detailed analysis of the actbl2 amino acids sequence with comparisons to other actin isoforms, please refer to Mazur et al. (2016).

Human *ACTBL2* (Gene Id: 345651), the gene encoding actbl2 (Uniprot accession number Q562R1), is located on chromosome 5 (5q11.2) and it is not a pseudogene. Under the Q562R1 number, an article (Chang et al., 2006) is cited that describes ϰ actin transcripts as a new actin family that was found in hepatocellular carcinoma. However, analysis of the nucleotide sequences mentioned in this article suggests that they are POTE-actin genes rather than at *ACTBL2*. This group of genes is found only in primates and is composed of an N-terminal cysteine-rich domain, a domain with ankyrin repeats, β actin, and a C-terminal domain containing spectrin-like helices. Products of these genes are 120 kDa proteins that are detected, for example, in breast cancer cell lines (Lee et al., 2006). POTE-actin genes have nothing in common with the *ACTBL2* gene.

Almost the only data suggesting the existence of actbl2 at the protein level was provided by mass spectrometry (MS) studies concerning predominantly posttranslational modifications such as SUMOylation (Golebiowski et al., 2009; Grant, 2010; Tatham et al., 2011) or ubiquitination (Vasilescu et al., 2007; Teixeira et al., 2010; Danielsen et al., 2011; Kim et al., 2011; Lopitz-Otsoa et al., 2012; Hanson et al., 2014; Leng et al., 2014), which are known to affect actins (Terman and Kashina, 2013). Unique actbl2 peptides identified in these studies are shown in Fig. 3. There are also MS-based studies suggesting that actbl2 is upregulated in colorectal (Ghazanfar et al., 2017) and pancreatic cancers (Kuwae et al., 2014). In our previous studies (Mazur et al., 2016), co-immunoprecipitates of gelsolin, an actin binding protein, were obtained from cell lysates, and nuclear fractions of melanoma cell lines were subjected to MS analysis, which revealed the presence of one specific actbl2 peptide in two separate experiments (Fig. 3). However, further analysis showed that *ACTBL2* is not expressed at the same high level as *ACTB*, the gene coding for β actin (Mazur et al., 2016).

There is only one publication that is currently available on the functional role of actbl2. Hoedebeck and colleagues (2014) showed that silencing *ACTBL2* led to decreased motility in human arterial smooth muscle cells. They also demonstrated that smooth muscle cell expression of ACTBL2 under stretching conditions is dependent on nuclear factor 5 in activated T-cells (NFAT5). In that study and a study conducted by Ghazanfar et al. (2016), rabbit polyclonal antibodies were used to detect actbl2. Because only a small number of amino acids differ between actins (Fig. 1), and because actbl2 is in 92% identical to β actin (Mazur et al., 2016), specificity of antibodies is critical while studying actins. This is why data should be interpreted

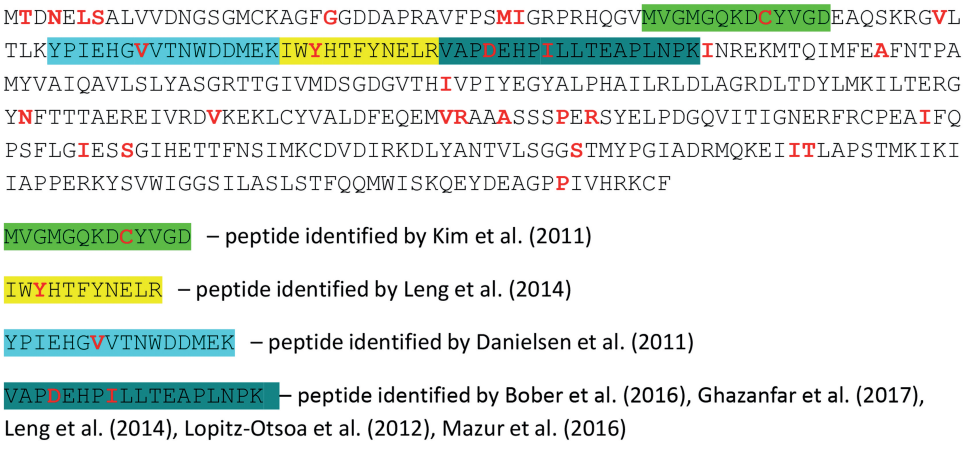

Fig. 3. Peptides that are unique for actbl2 were identified in different mass spectrometry-based studies. Actbl2-specific peptides are highlighted in colors. Unique amino acids for actbl2 differentiating it from the other six "classical" actins are marked in red.

with caution regardless of the immunohistochemical and western blot analyses shown by Ghazanfar et al. (2017) and (Hoedebeck et al., 2014).

Actbl2 can polymerize, as revealed by the analysis of A375 melanoma cells ectopically expressing HA-actbl2 (hemagglutinin tagged actbl2), because strong colocalization of signals from HA-actbl2 and F-actin (Mazur et al., 2016) and from HA-actbl2 and F-actin were visualized using LifeAct-TagRFP (Fig. 4A). F-actin with incorporated HA-actbl2 was present in lamellipodia, filopodia, and invadopodia (Fig. 4) (Mazur et al., 2016). A lack of HA-actbl2 colocalization with monomeric actin was observed (Fig. 4B). Intracellularly, both β and γ cytoplasmic actins are present as F- and G-actin. This suggests that actbl2 might be differentially regulated by actin binding proteins or that it polymerizes at a lower critical monomer concentration than "classical" cytoplasmic actins. While analyzing transfected cells, we also detected HA-actbl2 in the cell nuclei (Mazur et al., 2016).

There are at least two obstacles to studying actbl2. T, there have been no commercially available antibodies that will specifically recognize only actbl2. There are several polyclonal antibodies, which should recognize internal actbl2 epitopes, if it is indicated by the manufacturer. By having DNA construct coding for HA-actbl2, we used immunocytochemical and Western Blot applications to test some antibodies, and we found that those directed against internal actbl2 epitopes do not recognize HA-actbl2 and are not specific for actbl2 (Mazur, unpublished observations). Additionally, the *ACTBL2* gene is a single exon gene (SEG) with no introns (an intronless gene). In recent years, an increasing number of studies were published that focused on eukaryotic SEGs (Sakharkar et al., 2004; Hube and Francastel, 2015; Jorquera et al., 2016). Thus, while extracting mRNA from a sample, it is crucial to remove genomic DNA contaminations.

In summary, actbl2 studies should be planned carefully and high importance should be placed on removal of genomic contamination while performing rt-PCR, and on obtaining actbl2-specific monoclonal antibodies.

Concluding remarks

Using different approaches, β and γ non-muscle actins were shown to be only partly redundant and they display specific functions in the actin cytoskeletal structure organization (formation of stress fibres, actin networks, membrane protrusions), cell motility, gene transcription, cell division, and in developmental processes. However, it seems that for proper cell functioning, a balanced β-to-γ actin ratio and cooperation between both isoforms are required. Because of limited differences between β and γ actin amino acid sequences, it is possible that interactions of non-muscle isoactins with various actin binding proteins can help them to fulfill distinct functions within the organism.

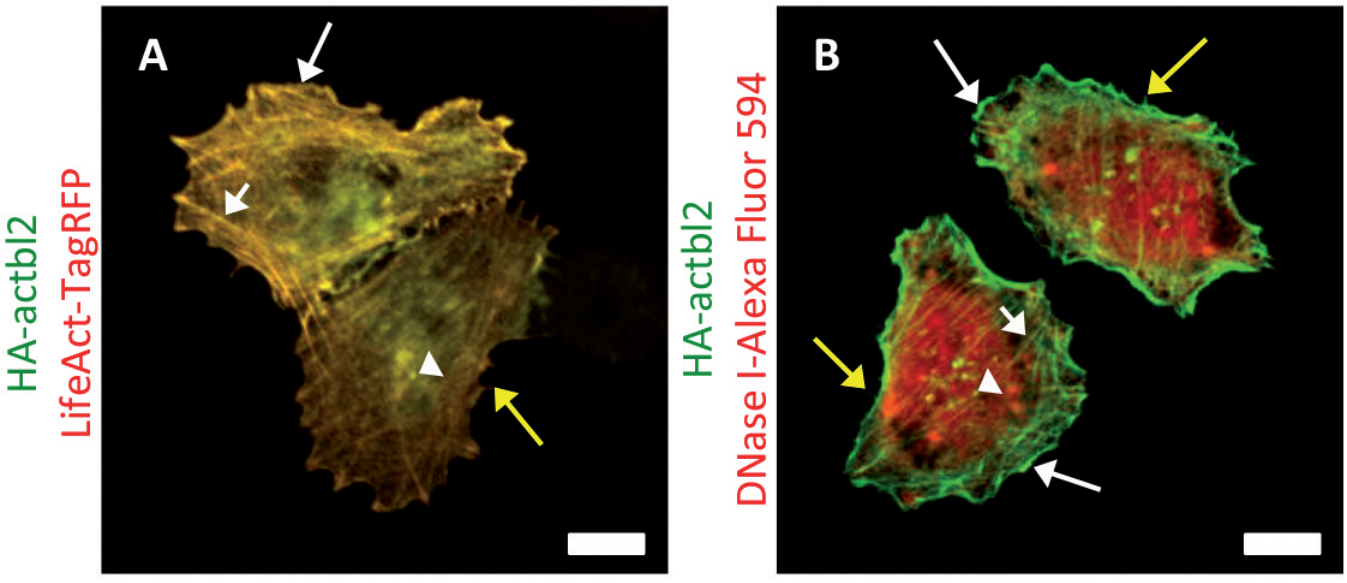

Fig. 4. Polymerization of actbl2. **A.** HA-actbl2 and LifeAct-TagRFP, a fluorescent F-actin marker expressed in A375 cells, were fixed and stained with anti-HA-Alexa Fluor 488 conjugated antibodies. **B.** A375 cells expressing hemagglutinin tagged actbl2 (HA-actbl2) were fixed and stained with anti-HA-Alexa Fluor 488 conjugated antibodies and DNase I-Alexa Fluor 594 to visualize G-actin. HA-actbl2 is present in the leading edge of lamellipodia (arrows), stress fibres (short arrows), filopodia (yellow arrows), invadopodia (arrowheads), and the cell nucleus (blue arrow). Scale bar: 20 μm.

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References

Ampe C., Libbrecht J. and Van Troys M. (2013). β-actin knock-out mouse embryonic fibroblasts show increased expression of LIM-, CH-, EFh-domain containing proteins with predicted common upstream regulators. Cytoskeleton 70, 766-774.

Ampe C. and Van Troys M. (2016). Mammalian actins: Isoform-specific functions and diseases. Handb. Exp. Pharmacol. pp 1-37.

Andrade L.R. (2015). Evidence for changes in beta- and gamma-actin proportions during inner ear hair cell life. Cytoskeleton 72, 282-291.

Arsenian S., Weinhold B., Oelgeschläger M., Rüther U. and Nordheim A. (1998). Serum response factor is essential for mesoderm formation during mouse embryogenesis. EMBO J. 17, 6289-6299.

Artman L., Dormoy-Raclet V., von Roretz C. and Gallouzi I-E. (2014). Planning your every move: The role of β-actin and its post-transcriptional regulation in cell motility. Semin. Cell Dev. Biol. 34, 33-43.

Bachvarova R., Cohen E.M., De Leon V., Tokunaga K., Sakiyama S. and Paynton B.V. (1989). Amounts and modulation of actin mRNAs in mouse oocytes and embryos. Development 106, 561-565.

Baranwal S., Naydenov N.G., Harris G., Dugina V., Morgan K.G., Chaponnier C. and Ivanov A.I. (2012). Nonredundant roles of cytoplasmic β- and γ-actin isoforms in regulation of epithelial apical junctions. Mol. Biol. Cell. 23, 3542-3553.

Belyantseva I.A., Perrin B.J., Sonnemann K.J., Zhu M., Stepanyan R., McGee J., Frolenkov G.I., Walsh E.J., Friderici K.H., Friedman T.B. and Ervasti J.M. (2009). Gamma-actin is required for cytoskeletal maintenance but not development. Proc. Natl. Acad. Sci. USA 106, 9703-9708.

Bergeron S.E., Zhu M., Thiem S.M., Friderici K.H. and Rubenstein P.A. (2010). Ion-dependent polymerization differences between mammalian beta- and gamma-nonmuscle actin isoforms. J. Biol. Chem. 285, 16087-16095.

Bober J., Olsnes S., Kostas M., Bogacz M., Zakrzewska M. and Otlewski J. (2016). Identification of new FGF1 binding partners - Implications for its intracellular function. IUBMB. Life 68, 242-251.

Brault V., Reedy M.C., Sauder U., Kammerer R.A., Aebi U. and Schoenenberger C. (1999). Substitution of flight muscle-specific actin by human (beta)-cytoplasmic actin in the indirect flight muscle of Drosophila. J. Cell. Sci. 112 Pt 2, 3627-3639.

Brockmann C., Huarte J., Dugina V., Challet L., Rey E., Conne B., Swetloff A., Nef S., Chaponnier C. and Vassalli J.D. (2011). Beta- and gamma-cytoplasmic actins are required for meiosis in mouse oocytes. Biol. Reprod. 85, 1025-1039.

Bulinski J.C. (2006). Cell biology. Actin discrimination. Science 313, 180-181.

Bunnell T.M. and Ervasti J.M. (2010). Delayed embryonic development and impaired cell growth and survival in Actg1 null mice. Cytoskeleton 67, 564-572.

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

Chang K.W., Yang P.Y., Lai H.Y., Yeh T.S., Chen T.C. and Yeh C.T. (2006). Identification of a novel actin isoform in hepatocellular carcinoma. Hepatol. Res. 36, 33-39.

Choidas A., Jungbluth A., Sechi A., Murphy J., Ullrich A. and Marriott G. (1998). The suitability and application of a GFP-actin fusion protein for long-term imaging of the organization and dynamics of the cytoskeleton in mammalian cells. Eur. J. Cell Biol. 77, 81-90.

Condeelis J. and Singer R.H. (2005). How and why does beta-actin mRNA target? Biol. Cell. 97, 97-110.

Cruz L.A., Vedula P., Gutierrez N., Shah N., Rodriguez S., Ayee B., Davis J. and Rodriguez A.J. (2015). Balancing spatially regulated β-actin translation and dynamin-mediated endocytosis is required to assemble functional epithelial monolayers. Cytoskeleton (Hoboken). 72, 597-608.

Danielsen J.M., Sylvestersen K.B., Bekker-Jensen S., Szklarczyk D., Poulsen J.W., Horn H., Jensen L.J., Mailand N. and Nielsen M.L. (2011). Mass spectrometric analysis of lysine ubiquitylation reveals promiscuity at site level. Mol. Cell Proteomics 10,M110.003590.

Di Donato N., Rump A., Koenig R., Der Kaloustian V.M., Halal F., Sonntag K., Krause C., Hackmann K., Hahn G., Schrock E. and Verloes A. (2014). Severe forms of Baraitser-Winter syndrome are caused by ACTB mutations rather than ACTG1 mutations. Eur. J. Hum. Genet. 22, 179-183.

Dormoy-Raclet V., Ménard I., Clair E., Kurban G., Mazroui R., Di Marco S., von Roretz C., Pause A. and Gallouzi I.E. (2007). The RNA-binding protein HuR promotes cell migration and cell invasion by stabilizing the beta-actin mRNA in a U-rich-element-dependent manner. Mol. Cell. Biol. 27, 5365-5380.

Dowling P., Maurya P., Meleady P., Glynn S.A., Dowd A.J., Henry M. and Clynes M. (2007). Purification and identification of a 7.6-kDa protein in media conditioned by superinvasive cancer cells. Anticancer Res. 27, 1309-1317.

Drummond M.C. and Friderici K.H. (2013). A novel actin mRNA splice variant regulates ACTG1 expression. PLoS Genet. 9, 1-10.

Dugina V., Zwaenepoel I., Gabbiani G., Clément S. and Chaponnier C. (2009). Beta and gamma-cytoplasmic actins display distinct distribution and functional diversity. J. Cell. Sci. 122, 2980-2988.

Dugina V., Khromova N., Rybko V., Blizniukov O., Shagieva G., Chaponnier C., Kopnin B. and Kopnin P. (2015). Tumor promotion by γ and suppression by β non-muscle actin isoforms. Oncotarget 6, 14556-14571.

Dugina V., Alieva I., Khromova N., Kireev I., Gunning P.W. and Kopnin P. (2016). Interaction of microtubules with the actin cytoskeleton via cross-talk of EB1-containing +TIPs and γ-actin in epithelial cells. Oncotarget 7, 18-20.

Farina K.L., Huttelmaier S., Musunuru K., Darnell R. and Singer R.H. (2003). Two ZBP1 KH domains facilitate beta-actin mRNA localization, granule formation, and cytoskeletal attachment. J. Cell Biol. 160, 77-87.

Franke W.W., Stehr S., Stumpp S., Kuhn C., Heid H., Rackwitz H.R., Schnölzer M., Baumann R., Holzhausen H.J. and Moll R. (1996). Specific immunohistochemical detection of cardiac/fetal alpha-actin in human cardiomyocytes and regenerating skeletal muscle cells. Differentiation 60, 245-250.

Furness D.N., Katori Y., Mahendrasingam S. and Hackney C.M. (2005). Differential distribution of beta- and gamma-actin in guinea-pig cochlear sensory and supporting cells. Hear. Res. 207, 22-34.

Ghazanfar S., Fatima I., Aslam M., Musharraf S.G., Sherman N.E., Moskaluk C., Fox J.W., Akhtar M.W. and Sadaf S. (2017). Identification of actin beta-like 2 (ACTBL2) as novel, upregulated

protein in colorectal cancer. J. Proteomics 152, 33-40.

Ghosh T., Soni K., Scaria V., Halimani M., Bhattacharjee C. and Pillai B. (2008). MicroRNA-mediated up-regulation of an alternatively polyadenylated variant of the mouse cytoplasmic beta-actin gene. Nucleic Acids Res. 36, 6318-6332.

Goidin D., Mamessier A., Staquet M.J., Schmitt D. and Berthier-Vergnes O. (2001). Ribosomal 18S RNA prevails over glyceraldehyde-3-phosphate dehydrogenase and beta-actin genes as internal standard for quantitative comparison of mRNA levels in invasive and noninvasive human melanoma cell subpopulations. Anal. Biochem. 295, 17-21.

Golebiowski F., Matic I., Tatham M.H., Cole C., Yin Y., Nakamura A., Cox J., Barton G.J., Mann M. and Hay R.T. (2009) System-wide changes to SUMO modifications in response to heat shock. Sci. Signal. 26, ra24.

Goujon M., McWilliam H., Li W., Valentin F., Squizzato S., Paern J. and Lopez R. (2010). A new bioinformatics analysis tools framework at EMBL-EBI. Nucleic Acids Res. 38, 695-699.

Grant M.M. (2010). Identification of SUMOylated proteins in neuroblastoma cells after treatment with hydrogen peroxide or ascorbate. BMB Rep. 43, 720-725.

Gurskaya N.G., Fradkov A.F., Pounkova N.I., Staroverov D.B., Bulina M.E., Yanushevich Y.G., Labas Y.A., Lukyanov S. and Lukyanov K.A. (2003) A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants. Biochem J. 373, 403-408.

Hanson D., Stevens A., Murray P.G., Black G.C. and Clayton P.E. (2014). Identifying biological pathways that underlie primordial short stature using network analysis. J. Mol. Endocrinol. 52, 333-344.

Harborth J., Elbashir S.M., Bechert K., Tuschl T. and Weber K. (2001). Identification of essential genes in cultured mammalian cells using small interfering RNAs. J. Cell Sci. 114, 4557-4565.

Hill M.A. and Gunning P. (1993). Beta and gamma actin mRNAs are differentially located within myoblasts. J. Cell Biol. 122, 825-832.

Hoedebeck M., Scherer C., Wagner A., Hecker M. and Korff T. (2014). TonEBP/NFAT5 regulates ACTBL2 expression in biomechanically activated vascular smooth muscle cells. Front. Physiol. 5, 1-8.

Höfer D., Ness W. and Drenckhahn D. (1997). Sorting of actin isoforms in chicken auditory hair cells. J. Cell Sci. 110, 765-770.

Hofmann W.A. (2009). Chapter 6 Cell and Molecular Biology of Nuclear Actin. Int. Rev. Cell. Mol. Biol. 273, 219-263.

Hofmann W.A., Arduini A., Nicol S.M., Camacho C.J., Lessard J.L., Fuller-Pace F.V. and de Lanerolle P. (2009). SUMOylation of nuclear actin. J. Cell Biol. 186, 193-200.

Hoock T.C., Newcomb P.M. and Herman I.M. (1991). Beta actin and its mRNA are localized at the plasma membrane and the regions of moving cytoplasm during the cellular response to injury. J. Cell Biol. 112, 653-664.

Hube F. and Francastel C. (2015) Mammalian introns: When the junk generates molecular diversity. Int. J. Mol. Sci. 16,4429-4452.

Hundt N., Preller M., Swolski O., Ang A.M., Mannherz H.G., Manstein D.J. and Müller M. (2014). Molecular mechanisms of disease-related human β-actin mutations p.R183W and p.E364K. FEBS J. 281, 5279-5291.

Hunter S., Jones P., Mitchell A., Apweiler R., Attwood T.K., Bateman A., Bernard T., Binns D., Bork P., Burge S., de Castro E., Coggill P., Corbett M., Das U., Daugherty L., Duquenne L., Finn R.D., Fraser M., Gough J., Haft D., Hulo N., Kahn D., Kelly E., Letunic I., Lonsdale D., Lopez R., Madera M., Maslen J., McAnulla C., McDowall J., McMenamin C., Mi H., Mutowo-Muellenet P., Mulder N., Natale D., Orengo C., Pesseat S., Punta M., Quinn A.F., Rivoire C., Sangrador-Vegas A., Selengut J.D., Sigrist C.J., Scheremetjew M., Tate J., Thimmajanarthanan M., Thomas P.D., Wu C.H., Yeats C. and Yong S.Y. (2012). InterPro in 2011: new developments in the family and domain prediction database. Nucleic Acids Res. 40, D306-312.

Hüttelmaier S., Zenklusen D., Lederer M., Dictenberg J., Lorenz M., Meng X., Bassell G.J., Condeelis J. and Singer R.H. (2005). Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. Nature 438, 512-515.

Jain R.K., Joyce P.B., Molinete M., Halban P.A. and Gorr S.U. (2001). Oligomerization of green fluorescent protein in the secretory pathway of endocrine cells. Biochem. J. 360, 645-649.

Jorquera R., Ortiz R., Ossandon F., Halban P.A. and Gorr S.U. (2016). SinEx DB: a database for single exon coding sequences in mammalian genomes. Database 2016, baw095.

Kabsch W., Mannherz H.G., Suck D., Pai E.F. and Holmes K.C. (1990). Atomic structure of the actin:DNase I complex. Nature 347, 37-44.

Karakozova M., Kozak M., Wong C.C.L., Bailey A.O., Yates J.R. 3rd, Mogilner A., Zebroski H. and Kashina A. (2006). Arginylation of beta-actin regulates actin cytoskeleton and cell motility. Science 313, 192-196.

Khaitlina S.Y. (2001). Functional specificity of actin isoforms. Int. Rev. Cytol. 202, 35-98.

Kashina A.S. (2006). Differential arginylation of actin isoforms: the mystery of the actin N-terminus. Trends Cell Biol. 16, 610-615.

Khaitlina S.Y. (2007). Mechanisms of spatial segregation of actin isoforms. Cell Tissue Biol. 1, 293-304.

Kim W., Bennett E.J., Huttlin E.L., Guo A., Li J., Possemato A., Sowa M.E., Rad R., Rush J., Comb M.J., Harper J.W. and Gygi S.P. (2011). Systematic and quantitative assessment of the ubiquitin-modified proteome. Mol. Cell 44, 325-340.

Kislauskis E.H., Zhu X., Singer R.H. (1997). beta-Actin messenger RNA localization and protein synthesis augment cell motility. J. Cell Biol. 136, 1263-1270.

Kuwae Y., Kakehashi A., Wakasa K. and Wei M. (2014). Paraneoplastic Ma antigen - Like 1 as a potential prognostic biomarker in human pancreatic ductal adenocarcinoma. Pancreas 44, 106-115.

Latham S.L., Chaponnier C., Dugina V., Couraud P.O., Grau G.E. and Combes V. (2013) Cooperation between β-and γ-cytoplasmic actins in the mechanical regulation of endothelial microparticle formation. FASEB J. 27, 672-683.

Le P.U., Nguyen T.N., Drolet-savoie P., Leclerc N. and Nabi I.R. (1998). Increased β -Actin Expression in an Invasive Moloney Sarcoma Virus-transformed MDCK Cell Variant Concentrates to the Tips of Multiple Pseudopodia. Cancer Res. 15, 1631-1635.

Lechuga S., Baranwal S., Li C., Naydenov N.G., Kuemmerle J.F., Dugina V., Chaponnier C. and Ivanov A.I. (2014). Loss of γ-cytoplasmic actin triggers myofibroblast transition of human epithelial cells. Mol. Biol. Cell. 25, 3133-3146.

Lee Y., Ise T., Ha D., Saint Fleur A., Hahn Y., Liu X.F., Nagata S., Lee B., Bera T.K. and Pastan I. (2006). Evolution and expression of chimeric POTE-actin genes in the human genome. Proc. Natl. Acad. Sci. USA 103, 17885-17890.

Leng L., Xu C., Wei C., Zhang J., Liu B., Ma J., Li N., Qin W., Zhang W., Zhang C., Xing X., Zhai L., Yang F., Li M., Jin C., Yuan Y., Xu P., Qin J., Xie H., He F. and Wang J. (2014). A proteomics strategy for the identification of FAT10-modified sites by mass spectrometry. J.

Proteome Res. 13, 268-276.

Lewis B.P., Green R.E. and Brenner S.E. (2003). Evidence for the widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans. Proc. Natl. Acad. Sci. USA 100, 189-192.

Lloyd C.M., Berendse M., Lloyd D.G., Schevzov G. and Grounds M.D. (2004). A novel role for non-muscle gamma-actin in skeletal muscle sarcomere assembly. Exp. Cell. Res. 297, 82-96.

Lopitz-Otsoa F., Rodriguez-Suarez E., Aillet F., Casado-Vela J., Lang V., Matthiesen R., Elortza F. and Rodriguez M.S. (2012). Integrative analysis of the ubiquitin proteome isolated using Tandem Ubiquitin Binding Entities (TUBEs). J. Proteomics 75, 2998-3014.

Mazur A.J., Radaszkiewicz T., Makowiecka A., Malicka-Błaszkiewicz M., Mannherz H.G. and Nowak D. (2016). Gelsolin interacts with LamR, hnRNP U, nestin, Arp3 and β-tubulin in human melanoma cells as revealed by immunoprecipitation and mass spectrometry. Eur. J. Cell Biol. 95, 26-41.

McDonald D., Carrero G., Andrin C., de Vries G. and Hendzel M.J. (2006). Nucleoplasmic beta-actin exists in a dynamic equilibrium between low-mobility polymeric species and rapidly diffusing populations. J. Cell Biol. 172, 541-552.

McHugh K.M., Crawford K. and Lessard J.L. (1991). A comprehensive analysis of the developmental and tissue-specific expression of the isoactin multigene family in the rat. Dev. Biol. 148, 442-458.

Migocka-Patrzałek M., Makowiecka A., Nowak D., Mazur A.J., Hofmann W.A. and Malicka-Błaszkiewicz M. (2015). β- and γ-Actins in the nucleus of human melanoma A375 cells. Histochem. Cell Biol. 144, 417-428.

Ming G-L. (2006). Turning by asymmetric actin. Nat. Neurosci. 9, 1201-1203.

Miyagawa M., Nishio S., Ichinose A., Iwasaki S., Murata T., Kitajiri S. and Usami S. (2015). Mutational spectrum and clinical features of patients with ACTG1 mutations identified by massively parallel DNA sequencing. Ann. Otol. Rhinol. Laryngol. 124, 84S-93S.

Morín M., Bryan K.E., Mayo-Merino F., Goodyear R., Mencía A., Modamio-Høybjør S., del Castillo I., Cabalka J.M., Richardson G., Moreno F., Rubenstein P.A. and Moreno-Pelayo M.A. (2009). *In vivo* and *in vitro* effects of two novel gamma-actin (ACTG1) mutations that cause DFNA20/26 hearing impairment. Hum. Mol. Genet. 18, 3075-3089.

Müller M., Diensthuber R.P., Chizhov I., Claus P., Heissler S.M., Preller M., Taft M.H. and Manstein D.J. (2013). Distinct functional interactions between actin isoforms and nonsarcomeric myosins. PLoS One 8, e70636.

Nagata K. and Ichikawa Y. (1984). Changes in actin during cell differentiation. Cell Muscle Motil. 5, 171-193.

Nowak D. and Malicka-Błaszkiewicz M. (1999). Actin isoforms--functional differentiation, changes in cell pathology. Postepy Biochem. 45, 261-269.

Nowak D., Skwarek-Maruszewska A., Zemanek-Zboch M. and Malicka-Błaszkiewicz M. (2005). Beta-actin in human colon adenocarcinoma cell lines with different metastatic potential. Acta Biochim. Pol. 52, 461-468.

Nunoi H., Yamazaki T., Tsuchiya H., Kato S., Malech H.L., Matsuda I. and Kanegasaki S. (1999). A heterozygous mutation of beta-actin associated with neutrophil dysfunction and recurrent infection. Proc. Natl. Acad. Sci. USA 96, 8693-8698.

Oleynikov Y. and Singer R.H. (2003) Real-time visualization of ZBP1 association with beta-actin mRNA during transcription and localization. Curr. Biol. 13, 199-207.

Otey C.A., Kalnoski M.H. and Bulinski J.C. (1987). Identification and quantification of actin isoforms in vertebrate cells and tissues. J. Cell Biochem. 34, 113-124.

Pasquier E., Tuset M-P., Sinnappan S., Carnell M., Macmillan A. and Kavallaris M. (2015). γ-Actin plays a key role in endothelial cell motility and neovessel maintenance. Vasc. Cell 7, 1-16.

Pavlyk I., Rzhepetskyy Y., Jagielski A.K., Drozak J., Wasik A., Pereverzieva G., Olchowik M., Kunz-Schugart L.A., Stasyk O. and Redowicz M.J. (2015). Arginine deprivation affects glioblastoma cell adhesion, invasiveness and actin cytoskeleton organization by impairment of β-actin arginylation. Amino Acids 47, 199-212.

Peckham M., Miller G., Wells C., Zicha D. and Dunn G.A. (2001). Specific changes to the mechanism of cell locomotion induced by overexpression of beta-actin. J. Cell Sci. 114, 1367-1377.

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

Pinder J.C. and Gratzer W.B. (1983). Structural and dynamic states of actin in the erythrocyte. J. Cell Biol. 96, 768-775.

Po'uha S.T. and Kavallaris M. (2015). Gamma-actin is involved in regulating centrosome function and mitotic progression in cancer cells. Cell Cycle 14, 3908-3919.

Po'uha S.T., Honore S., Braguer D. and Kavallaris M. (2013). Partial depletion of gamma-actin suppresses microtubule dynamics. Cytoskeleton 70, 148-160.

Pollard T.D. (2016). Actin and actin-binding proteins. Cold Spring Harb. Perspect. Biol. 8, a018226.

Pollard T.D. and Borisy G.G. (2003). Cellular motility driven by assembly and disassembly of actin filaments. Cell 112, 453-465.

Posern G. and Treisman R. (2006). Actin' together: serum response factor, its cofactors and the link to signal transduction. Trends Cell Biol. 16, 588-596.

Posern G., Sotiropoulos A. and Treisman R. (2002). Mutant actins demonstrate a role for unpolymerized actin in control of transcription by serum response factor. Mol. Biol. Cell.13, 4167-4178.

Prins K.W., Lowe D.A. and Ervasti J.M. (2008). Skeletal muscle-specific ablation of gamma(cyto)-actin does not exacerbate the mdx phenotype. PLoS One 3,e2419.

Procaccio V., Salazar G., Ono S., Styers M.L., Gearing M., Davila A., Jimenez R., Juncos J., Gutekunst C.A., Meroni G., Fontanella B., Sontag E., Sontag J.M., Faundez V. and Wainer B.H. (2006). A mutation of beta -actin that alters depolymerization dynamics is associated with autosomal dominant developmental malformations, deafness, and dystonia. Am. J. Hum. Genet. 78, 947-960.

Rivière J., van Bon B.W., Hoischen A., Kholmanskikh S.S., O'Roak B.J., Gilissen C., Gijsen S., Sullivan C.T., Christian S.L., Abdul-Rahman O.A., Atkin J.F., Chassaing N., Drouin-Garraud V., Fry A.E., Fryns J.P., Gripp K.W., Kempers M., Kleefstra T., Mancini G.M., Nowaczyk M.J., van Ravenswaaij-Arts C.M., Roscioli T., Marble M., Rosenfeld J.A., Siu V.M., de Vries B.B., Shendure J., Verloes A., Veltman J.A., Brunner H.G., Ross M.E., Pilz D.T. and Dobyns W.B. (2013). De novo mutations in the actin genes ACTB and ACTG1 cause Baraitser-Winter syndrome. Nat. Genet. 26, 440-444.

Rommelaere H., Waterschoot D., Neirynck K., Vandekerckhove J. and Ampe C. (2004). A method for rapidly screening functionality of actin mutants and tagged actins. Biol. Proced. Online 6, 235-249.

Ross A.F., Oleynikov Y., Kislauskis E.H. and Taneja K.L. (1997). Characterization of a beta-actin mRNA zipcode-binding protein. Mol. Cell Biol. 17, 2158-2165.

Rould M.A., Wan Q., Joel P.B., Lowey S. and Trybus K.M. (2006). Crystal structures of expressed non-polymerizable monomeric actin in the ADP and ATP states. J. Biol. Chem. 281, 31909-31919.

Rubenstein P.A. and Wen K.K. (2014). Insights into the effects of disease-causing mutations in human actins. Cytoskeleton 71, 211-229.

Rybakova I.N., Patel J.R. and Ervasti J.M. (2000). The dystrophin complex forms a mechanically strong link between the sarcolemma and costameric actin. J. Cell Biol. 150, 1209-1214.

Sakharkar M.K., Chow V.T., Chaturvedi I., Mathura V.S., Shapshak P. and Kangueane P. (2004). A report on single exon genes (SEG) in eukaryotes. Front. Biosci. 9, 3262-3267.

Schevzov G., Lloyd C. and Gunning P. (1992). High level expression of transfected beta- and gamma-actin genes differentially impacts on myoblast cytoarchitecture. J. Cell Biol. 117, 775-785.

Shagieva G.S., Domnina L.V., Chipysheva T., Ermilova V.D., Chaponnier C. and Dugina V.B. (2012). Actin isoforms and reorganization of adhesion junctions in epithelial-to-mesenchymal transition of cervical carcinoma cells. Biochemistry (Mosc.) 77, 1266-1276.

Shestakova E.A., Wyckoff J., Jones J., Singer R.H. and Condeelis J. (1999). Correlation of beta-actin messenger RNA localization with metastatic potential in rat adenocarcinoma cell lines. Cancer Res. 59, 1202-1205.

Sheterline P., Clayton J. and Sparrow J. (1995). Actin. Protein Profile 2, 1-103.

Shmerling D., Danzer C-P., Mao X., Boisclair J., Haffner M., Lemaistre M., Schuler V., Kaeslin E., Korn R., Bürki K., Ledermann B., Kinzel B. and Müller M. (2005) Strong and ubiquitous expression of transgenes targeted into the beta-actin locus by Cre/lox cassette replacement. Genesis 42, 229-235.

Shum M.S., Pasquier E., Po'uha S.T., O'Neill G.M., Chaponnier C., Gunning P.W. and Kavallaris M. (2011) γ-Actin regulates cell migration and modulates the ROCK signaling pathway. FASEB J. 25, 4423-4433.

Sigrist C.J.A., De Castro E., Cerutti L., Cuche B.A., Hulo N., Bridge A., Bougueleret L. and Xenarios I. (2013). New and continuing developments at PROSITE. Nucleic Acids Res. 41, 344-347.

Simiczyjew A., Mazur A.J., Popow-Woźniak A., Malicka-Blaszkiewicz M. and Nowak D. (2014). Effect of overexpression of β- and γ-actin isoforms on actin cytoskeleton organization and migration of human colon cancer cells. Histochem. Cell Biol. 142, 307-322.

Simiczyjew A., Mazur A.J., Ampe C., Malicka-Błaszkiewicz M., van Troys M. and Nowak D. (2015). Active invadopodia of mesenchymally migrating cancer cells contain both β and γ cytoplasmic actin isoforms. Exp. Cell Res. 339, 206-219.

Song T., Zheng Y., Wang Y., Katz Z., Liu X., Chen S., Singer R.H. and Gu W. (2015). Specific interaction of KIF11 with ZBP1 regulates the transport of β-actin mRNA and cell motility. J. Cell Sci. 128, 1001-1010.

Sotiropoulos A., Gineitis D., Copeland J. and Treisman R. (1999). Signal-regulated activation of serum response factor is mediated by changes in actin dynamics. Cell 98, 159-169.

Stöhr N. and Hüttelmaier S. (2012) IGF2BP1: a post-transcriptional "driver" of tumor cell migration. Cell Adh. Migr. 6, 312-318.

Tatham M.H., Matic I., Mann M. and Hay R.T. (2011). Comparative proteomic analysis identifies a role for SUMO in protein quality control. Sci. Signal. 4, rs4.

Teixeira F.R., Yokoo S., Gartner C., Manfiolli A.O., Baqui M.M., Assmann E.M., Maragno A.L., Yu H., de Lanerolle P., Kobarg J., Gygi S.P. and Gomes M.D. (2010). Identification of FBXO25-interacting proteins using an integrated proteomics approach. Proteomics 10, 2746-2757.

Terman J.R. and Kashina A. (2013). Post-translational modification and regulation of actin. Curr. Opin. Cell Biol. 25, 30-38.

Tondeleir D., Drogat B., Slowicka K., Bakkali K., Bartunkova S., Goossens S., Haigh J.J. and Ampe C. (2013). Beta-actin is involved in modulating erythropoiesis during development by fine-tuning Gata2 expression levels. PLoS One. 8, e67855.

Tondeleir D., Lambrechts A., Müller M., Jonckheere V., Doll T., Vandamme D., Bakkali K., Waterschoot D., Lemaistre M., Debeir O., Decaestecker C., Hinz B., Staes A., Timmerman E., Colaert N., Gevaert K., Vandekerckhove J. and Ampe C. (2012). Cells lacking β-actin are genetically reprogrammed and maintain conditional migratory capacity. Mol. Cell Proteomics 11, 255-271.

Tzima E., Trotter P.J., Orchard M.A. and Walker J.H. (2000). Annexin V relocates to the platelet cytoskeleton upon activation and binds to a specific isoform of actin. Eur. J. Biochem. 267, 4720-4730.

van Wijk E., Krieger E., Kemperman M.H., De Leenheer E.M., Huygen P.L., Cremers C.W., Cremers F.P. and Kremer H. (2003). A mutation in the gamma actin 1 (ACTG1) gene causes autosomal dominant hearing loss (DFNA20/26). J. Med. Genet. 40, 879-884.

Vandekerckhove J. and Weber K. (1978). At least six different actins are expressed in a higher mammal: an analysis based on the amino acid sequence of the amino-terminal tryptic peptide. J. Mol. Biol. 126, 783-802.

Vandekerckhove J. and Weber K. (1981). Actin typing on total cellular extracts: a highly sensitive protein-chemical procedure able to distinguish different actins. Eur. J. Biochem. 113, 595-603.

Vasilescu J., Zweitzig D.R., Denis N.J., Smith J.C., Ethier M., Haines D.S. and Figeys D. (2007). The proteomic reactor facilitates the analysis of affinity-purified proteins by mass spectrometry: Application for identifying ubiquitinated proteins in human cells. J. Proteome Res. 6, 298-305.

Verrills N.M., Po'uha S.T., Liu M.L., Liaw T.Y., Larsen M.R., Ivery M.T., Marshall G.M., Gunning P.W. and Kavallaris M. (2006). Alterations in gamma-actin and tubulin-targeted drug resistance in childhood leukemia. J. Natl. Cancer Inst. 98,1363-1374.

Virtanen J.A. and Vartiainen M.K. (2017). Diverse functions for different forms of nuclear actin. Curr. Opin. Cell Biol. 46, 33-38.

Zhang F., Saha S., Shabalina S. and Kashina A. (2010). Differential arginylation of actin isoforms is regulated by coding sequence-dependent degradation. Science 329, 1534-1537.

Zheng B., Han M., Bernier M. and Wen J. (2009). Nuclear actin and actin-binding proteins in the regulation of transcription and gene expression. FEBS J. 276, 2669-2685.

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