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

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

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 Mg²⁺ 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 acts (like mutations, changes in gene expression or cellular level, polymerization status) is still growing (Nunoo et al., 1999; Proacco et al., 2006; Rivières 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-Blaszkiewicz, 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 paclitaxel 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 synthesize 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 depolymerization by coflin. 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.

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>Effect of mutation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils, a point mutation in β actin gene (Glu364Lys)</td>
<td>Reduced chemotactic cell response and reduced peroxide forming ability; resulting in mental disability, sensitivity to light, prone to infections</td>
<td>Nunoi et al., 1999; Hundt et al., 2014</td>
</tr>
<tr>
<td>Point mutation in β actin gene (Arg183Trp)</td>
<td>Formation of stable filaments, lack of depolymerization; resulting in distortion during fetal development, hearing impairment, dystonia</td>
<td>Procaccio et al., 2006; Hundt et al., 2014</td>
</tr>
<tr>
<td>Point mutation in γ actin gene (Thr278Ile)</td>
<td>Weakness of filaments, disorders in stereocilia renewal; resulting in hearing loss</td>
<td>van Wijk et al., 2003</td>
</tr>
<tr>
<td>Mutations in the γ actin gene, which reduce the level of γ actin (Val103Leu, Asp187His, Thr162Met, Pro98Leu)</td>
<td>Acute lymphoblastic leukemia is less sensitive to agents targeting microtubules</td>
<td>Verrills et al., 2006</td>
</tr>
<tr>
<td>Point mutation in γ actin gene, DFNA20/26 (Lys118Asn; Glu241Lys; Gly48Arg; Leu229Val)</td>
<td>Hearing impairment connected to autosomal dominant sensorineural hearing loss</td>
<td>Morín et al., 2009; Miyagawa et al., 2015</td>
</tr>
<tr>
<td>Point mutations in β actin gene (Arg196His) and γ actin gene (Ser155Phe)</td>
<td>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</td>
<td>Rivière et al., 2013; Di Donato et al., 2014</td>
</tr>
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</table>
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 acts 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-Patrzafek 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 (Simiczew 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, coflin1, 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 synthesized 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 they 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-terminal area of the polypeptide chain. An arginine residue on the N-terminal 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 (Karazkova 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 (Karazkova 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; Karazkova 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 posttranslational modifications such as acetylation, methylation, oxidation, phosphorylation, and ubiquitination (Terman and Kashina, 2013). It was also shown that nuclear 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 is much more 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-terminal area of the polypeptide chain. An arginine residue on the N-terminal 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 (Karazkova 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 (Karazkova 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; Karazkova 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).
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.

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

**Actb2, another actin isoform?**

β actin-like protein 2 (Actb2) is 92% identical to β actin. Actb2 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 actb2 amino acids sequence with comparisons to other actin isoforms, please refer to Mazur et al. (2016).

Human ACTBL2 (Gene Id: 345651), the gene encoding actb2 (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 Actb2. 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 actb2 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-Ortsoa et al., 2012; Hanson et al., 2014; Leng et al., 2014), which are known to affect actins (Terman and Kashina, 2013). Unique actb2 peptides identified in these studies are shown in Fig. 3. There are also MS-based studies suggesting that actb2 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 actb2 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 actb2. 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 actb2. Because only a small number of amino acids differ between actins (Fig. 1), and because actb2 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 actb2 were identified in different mass spectrometry-based studies. Actb2-specific peptides are highlighted in colors. Unique amino acids for actb2 differentiating it from the other six “classical” actins are marked in red.](image-url)
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. 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.
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References


Equality of non-muscle acts

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Equality of non-muscle actins


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