TOURO COLLEGE & UNIVERSITY SYSTEM

The Science Journal of the Lander College of Arts and Sciences

Volume 7
Number 1 *Fall 2013*

1-1-2013

Cytoplasmic Actin: Structure and Function

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Abstract

Cytoplasmic actin plays a crucial role in cellular structure, cell motility, intracellular transportation, and the cell cycle. Two isoforms of cytoplasmic actin have been identified, β and γ. Although their amino acid sequence is nearly identical, these two isoforms are encoded by different genes located on different chromosomes. Recent research has found that, despite their similarities, the two isoforms of cytoplasmic actin have distinct functions. This paper will review the structural and functional differences between the two isoforms, concluding with a discussion of some mutations that have been linked to disease.

Introduction

Actin is one of the most abundant proteins, accounting for 10% of protein content in muscular cells, and 1-5% of protein content in all other cells. Muscular actin, together with myosin, is responsible for the contraction of muscles. Cytoplasmic actin functions as a part of the cytoskeleton, and plays a role in cellular structure and motility, cytokinesis, phagocytosis, and intracellular transportation (Lodish et. al. 2013). Although it may have been observed as early as 1887 by W.D. Halliburton, actin was not purified in significant quantities until 1942 when Brunó Ferenc Straub developed a new technique for extracting muscle proteins. Soon after Straub's discovery, researchers reported finding actin in non-muscle cells. These findings were confirmed by Sadashi Hatano in 1968 (Schleicher and Jockusch, 2008). Since then, technological innovations have allowed researchers to solve its amino acid sequence and 3D structure.

Two isoforms of cytoplasmic actin have been identified, β and γ (also referred to as cytoplasmic actin 1 and 2, respectively). Although their amino acid sequence is nearly identical, these two isoforms are encoded by different genes located on different chromosomes; the gene for β actin is located on chromosome 7p ("Actin, Beta"); the gene for γ actin is located on chromosome 17q ("Actin, Gamma 1"). Both β and γ actin are necessary for proper cellular function. Mice in which the gene for β actin was erased on both chromosomes (actin knockout mice) died during early development. In contrast, mice engineered not to express γ actin are viable; however, they are smaller than the wild type and they experience a shortened lifespan. In humans, deficiencies in γ actin particularly affect the sensory hair cells of the inner ear. Scientists have proposed two theories to explain the distinct functions of β and γ actin. First, a number of actin binding proteins bind specifically to one isoform. The second theory is that the difference in function is due to the localization of the different isoforms by various cellular mechanisms. There is research that supports both theories (Perrin and Ervasti, 2010).

Structure

Quaternary Structure

Actin is found in cells as globular monomers (G-actin) and as filamentous polymers (F-actin). These filaments are made up of two strands of F-actin subunits wound around each other in a helical formation. Each filament has a (+) end and a (-) end, also referred to as the barbed end (+) and the pointed end (-). The (+) end polymerizes ten times quicker than the (-) end, and the (-) end de-polymerizes a little quicker. These filaments, called microfilaments, are highly organized and form part of the cytoskeleton. The organization of actin filaments in the cytoplasm differs from cell to cell, and is regulated by a number of proteins (Lodish et. al. 2013).

Actin is often associated with other molecules, most notably ATP and a divalent cation (usually Mg^{2+} or Ca^{2+}). Binding to ATP and Mg2+ changes the conformation of actin and is crucial to its polymerization. Myosin is another protein that is often associated with actin. Muscular actin combines with myosin to form contractile units called sarcomeres. In the cytoplasm, myosin motor proteins transport vesicles by moving along the actin microfilaments. There are also a number of proteins collectively referred to as actin binding proteins (ABPs) that bind to actin and regulate its polymerization and organization (Lodish et. al., 2013; Dominguez and Holmes, 2011).

Tertiary Structure

Actin is a globular protein that is divided by a cleft into two lobes. The upper cleft is the main binding site for ATP and Mg^{2+} ions; the lower part, lined with hydrophobic residues, is the main binding site for actin binding proteins (ABPs) (Domiguez ans Holmez, 2011). The molecule is further divided into four subunits numbered 1-4 (the bottom two domain are 1 & 3; the top two are 2 & 4). Both the N-teminus and the C-terminus ends are located in domain 1 (Lodish et. al. 2013). Domain 2, and particularly the DNase 1 - binding loop located in domain 2, play a critical role in polymerization. There is a small difference in conformation between G-actin and F-actin subunits. In G-actin domains 1 and 3 are rotated approximately 20º whereas F-actin subunits are flatter (Dominguez and Holmes, 2011).

Secondary Structure

SOPMA software predicts the following secondary structure for cytoplasmic actin 1 (β): alpha helix 33.60%, extended strand 22.40%, beta turn 6.67% and random coil 37.33%. The secondary structure for cytoplasmic actin 2 (γ) is as follows: alpha helix 34.67%, extended strand, 24.00%, beta turn 7.47%, random coil 33.87%. (Jonnalagedda et.al. 2012)

Primary Structure

Cytoplasmic actin 1 is comprised of 375 amino acids). It has an estimated molecular weight of 41,736g, and it is slightly

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acidic, with a pI of 5.29. It is noteworthy that actin has very high concentrations of glycine (7.5%) and proline (5.1%). This characteristic contributes to actin's compact structure. The different isoforms of human actin have a nearly identical amino acid sequence. Cytoplasmic actin 2, also made up of 375 amino acids, differs from cytoplasmic actin 1 in only four residues (2, 3, 4 & 9). Its PI is estimated to be 5.31, and it has an estimated molecular weight of 41,792.8g. α skeletal actin contains 377 amino acids. It differs from the cytoplasmic actins in only 25 residues, and 15 of those are considered to be similar (The UniProt Consortium, 2013, Jonnalagedda et. al., 2012).

Actin is highly conserved across different species. The actin found in the fungus Saccharomyces cerevisiae (baker's yeast) closely resembles cytoplasmic actin found in humans. It is also made of 375 amino acids, and it differs in only 42 of the residues, 30 of which are considered to be similar. The UniProt Consortium). S. cerevisiae actin has a MW of 41,689g, and a pI of 5.44, similar to that of human actin. (The secondary structure of S. cerevisiae actin is also very similar to human actin: alpha helix 33.87%, extended strand 24.00%, beta turn 6.93% and random coil 35.20%.

Functional Differences Between β and γ Actin

The amino acid sequences of the two isoforms are nearly identical; they differ in only four residues (2, 3, 4 and 9) (Figure 1). Interestingly, although their amino acid sequences are nearly identical, there are significant differences between the 5′ and 3′ un-translated regions surrounding the genes; these differences are conserved among species (Erba et-al). The gene encoding β actin is located on chromosome 7p; the gene encoding γ actin is located on chromosome 17q.

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MDDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQS
MEEEIAALVIDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQS
*:::*****:**************************************************
KRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMT
KRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMT
************************************************************
QIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGYALPHAILRLDL
QIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGVTHTVPIYEGYALPHAILRLDL
************************************************************
AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSY
AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSY
************************************************************
ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTFNSIMKCDVDIRKDLYANTVLS
ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTFNSIMKCDVDIRKDLYANTVLS
************************************************************
GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQ
GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWISKQ
************************************************************
EYDESGPSIVHRKCF
EYDESGPSIVHRKCF
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Figure 1: A comparison of the amino acid sequences of β and γ cytoplasmic actin. The top line is β actin; the bottom line is γ actin. The residues that differ have been highlighted. (The (:) symbol beneath these residues indicates that they are conserved mutations) (GeneCards.org).

Experimental evidence suggests that the two isoforms of cytoplasmic actin have some overlapping functions, but are not interchangeable. In γ actin knockout mice it was found that cellular concentrations of actin were normal, indicating that the body compensates for deficiencies in γ actin by up-regulating the expression of β actin. However, despite the fact that the total amount of actin was normal, these mice were smaller than the wild type, and significant numbers died due to developmental delays. In contrast to γ actin knockout mice, β actin knockout mice are not viable, demonstrating β actin's greater role in critical cellular functions (Perrin and Ervasti, 2010).

Experiments have shown that the relative concentrations of actin differ between cell types. Generally, β actin is present in greater concentrations than γ actin. However, in the cilia of the inner ear and epithelial cells of the intestinal tract γ actin dominates (Zhu et. al, 2003; Rendtorff et. al., 2006). Even within cells different isoforms of actin seem to be localized to specific areas. The mechanism that controls the localization of the different isoforms has not been identified. It is likely that the concentration in different cell types is regulated by the 5′ and 3′ UTRs, which regulate gene expression. As previously mentioned, significant differences have been found in the UTRs of the two genes. Differences in localization within the cell may be the result of differences in interactions with actin binding proteins (Perrin and Ervasti, 2010).

The differences in function between the two isoforms of cytoplasmic actin may be due to differences in the rate of polymerization. Under calcium-bound conditions β actin polymerized and de-polymerized at a quicker rate than γ actin. β actin and γ actin readily copolymerize, and the relative rates of polymerization of different filaments vary according to the ratio of the two isoforms. Because they polymerize and de-polymerize at a slower rate, filaments containing a higher ratio of gamma actin are more stable. The reason for these differences is unclear. One theory supported by research is that certain actin binding proteins that regulate polymerization are isoform-specific (Perrin and Ervasti, 2010).

Pathology

Hearing Loss

The function of the inner ear relies on the specialized structure of the auditory hair cell, and the function of these cells is closely related to the structure of their cytoskeleton. Mutations in cytoplasmic actin which result in malformations of the cytoskeleton have been found to cause hearing loss. These mutations affect the shape of the actin monomer, the stability of actin filaments, and actin's ability to bind to other proteins. Subtle differences in the onset and severity of the hearing loss have been observed in patients carrying different mutations (Zhu et. al..2003; Morin et. al.,2009). In one family a genetic mutation that causes isoleucine to be incorporated in place of threonine at position 278 (T278I) was observed. This is a non-conserved mutation; threonie is hydrophilic, and isoleucine is larger and hydrophobic. This mutation affects the 9 and 11 helices, structures that play a role in polymerization (Figure 2) (Van Wijk et. al, 2003).

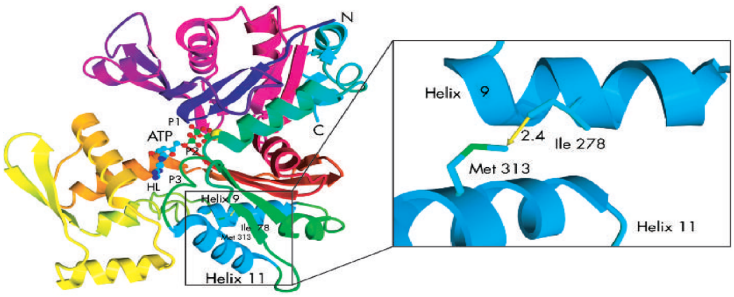
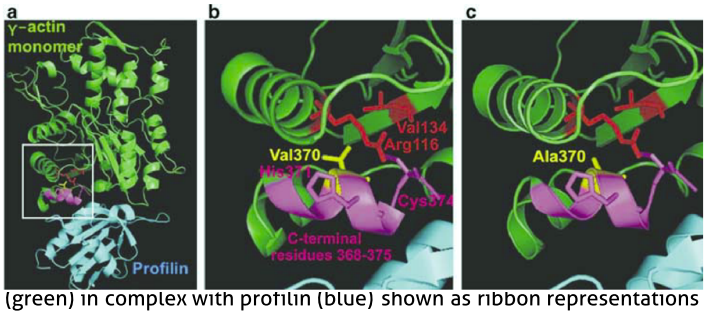


Figure 2: Structure of actin from D. discoideum. The protein is colored with a gradient from blue (N-terminus) to cyan (C-terminus), except for helices 9 and 11, which are shown in light blue. The three ATP binding loops (P1 to P3) and the hydrophobic loop involved in polymerization (HL) are marked. At position 278, the predicted side chain conformation of the isoleucine mutant is indicated, causing a strong bump with Met 313 (distance is 2.4 A°). The image was created with YASARA (www.yasara.org) (Van Wijk et. al., 2003).

Another mutation that has been studied is caused by the substitution of valine for alanine at position 370 (V370A). The valine at position 370 engages in hydrophobic interactions with neighboring side chains, stabilizing the C-terminal tail, a region that plays a critical role in the binding of some APBs. Alanine's side chain is too short to establish these interactions, destabilizing the C-terminal tail (Figure 3) (Rendtorff et.al., 2006).



(green) in complex with profilin (blue) shown as ribbon representations with side chains of residues discussed in the text. V370 is shown in yellow and the C-terminus of g-actin and residues herein that interact with V370 in magenta. Residues elsewhere in g-actin that interact with V370 are shown in red. (b) Enlarged view of the boxed region in (a). (c) As in (b), except that g-actin is the p.V370A mutant (Rendtorff et. al., 2006).

It is interesting to note that although γ actin is the prevalent isoform in the epithelial cells of the intestinal tract, these mutations have not been linked to any pathology of the intestines. This may be due to the fact that the cilia of the inner ear are subjected to high amount of mechanical trauma making them more susceptible to structural damage. Additionally, the cells of the intestinal tract are frequently renewed, making them less susceptible to such damage (Zhu et. al., 2003; Rendtorf et. al., 2006).

Muscular Disorders

Although muscular actin is the predominant isoform in muscle cells, mutations in cytoplasmic actin have also been linked to muscular disorders. One mutation in β actin that substitutes tryptophan for arginine at position 183 (R183W) has been linked to delayed onset dystonia. The mutated actin is more acidic than the wild type (PI 5.21 instead of 5.29), and slows depolymerization, making the actin filaments more rigid. This mutation has also been linked to hearing loss. Mutations in β actin have been linked to other muscular disorders as well, including, actin myopathy, nemaline myopathy, and intranuclear rod myopathy (Procaccio et. al., 2006).

Baraitser-Winter Syndrome

Mutations in both β and γ actin genes have been identified in patients with Baraitser-Winter Syndrome, a rare neurological disease. The two mutations that appeared most often caused a substitution of histidine in place of arginine at position 196 in β actin, and a substitution of serine for phenylalanine at position 155 in γ actin. The fact that mutations in both isoforms have been implicated suggests that the mutation affects a function that is common to both isoforms (Riviere et. al., 2013).

Conclusion

Cytoplasmic actin plays a crucial role in cellular structure, cell motility, intracellular transportation, and the cell cycle. As such, mutations in cytoplasmic actin can have wide-ranging deleterious effects on the organism. Because of their close similarity, researchers have had trouble identifying the functions that are unique to the different isoforms. However, it is clear at despite their overlapping functions the two isoforms are not interchangeable.

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