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Actinous enigma or enigmatic actin

Folding, structure, and functions of the most abundant eukaryotic protein

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Being the most abundant protein of the eukaryotic cell, actin continues to keep its secrets for more than 60 years. Everything about this protein, its structure, functions, and folding, is mysteriously counterintuitive, and this review represents an attempt to solve some of the riddles and conundrums commonly found in the field of actin research. In fact, actin is a promiscuous binder with a wide spectrum of biological activities. It can exist in at least three structural forms, globular, fibrillar, and inactive (G-, F-, and I-actin, respectively). G-actin represents a thermodynamically instable, quasi-stationary state, which is formed in vivo as a result of the energy-intensive, complex posttranslational folding events controlled and driven by cellular folding machinery. The G-actin structure is dependent on the ATP and Mg^{2+} binding (which in vitro is typically substituted by Ca^{2+}) and protein is easily converted to the I-actin by the removal of metal ions and by action of various denaturing agents (pH, temperature, and chemical denaturants). I-actin cannot be converted back to the G-form. Foldable and "natively folded" forms of actin are always involved in interactions either with the specific protein partners, such as Hsp70 chaperone, prefoldin, and the CCT chaperonin during the actin folding in vivo or with Mg^{2+} and ATP as it takes place in the G-form. We emphasize that the solutions for the mysteries of actin multifunctionality, multistructurality, and trapped unfolding can be found in the quasi-stationary nature of this enigmatic protein, which clearly possesses many features attributed to both globular and intrinsically disordered proteins.

Introduction

Actin is the most abundant protein in the eukaryotic cells, where the monomeric actin concentrations are within the 12–300 μM range.[1] Being found in almost every living cell, this globular multifunctional protein is most common in the muscle cells, where its concentration ranges from 230–960 μM.[1] Among various functional and structural features of actin are its ability to exist as a monomer known as G-actin (under low ionic strength conditions) or a single-stranded polymer, the so-called fibrous form of actin, or F-actin (which results from the polymerization of G-actin in the presence of neutral salts), or an inactive form that lacks the ability to polymerize and can be produced by the release of cations by EDTA or EGTA treatment[2-4] among other means. All this defines the constant interest of researchers to this enigmatic protein. In fact, actin, the muscle form of which was discovered more than 60 years ago,[5] continues to be a subject of very intensive research (see **Fig. 1**).

The primary focus of early actin-related studies was on the ability of this protein to polymerize and to interact with the other main muscle proteins, such as myosin, as well as with the regulatory proteins controlling muscle relaxation and contraction.[6] The role of ATP hydrolysis as a source of energy and the role of Mg^{2+} (which is replaced by Ca^{2+} in vitro) as an essential component of the polymerization-depolymerization process have been studied as well.[7] Subsequent studies were focused on the roles of actin in non-muscle cells, particularly on the involvement of this protein in the formation of the cytoskeleton that functions to enable cell motility and inter-cell interaction.[1,8,9] Further investigations showed that actin participates in many crucial cellular processes, such as endocytosis and intracellular trafficking.[10,11] Although the nuclear localization of actin was reported almost at the same time as the discovery of this protein in the cytoplasm,[12] for a long time it was taken as an artifact.[13] However, recent studies provided solid support to the idea that actin is as important in nucleus as it does in the cytoplasm, and established that actin has numerous roles in the cell nucleus, starting from the formation of the nuclear scaffold and ending with various roles of the G-actin in transcription and chromatin remodeling.[14-17]

Therefore, all these data show that actin is involved in interaction with a large number of unrelated proteins in different cell compartments. This binding promiscuity is one of the characteristic features of intrinsically disordered proteins (IDPs) in general and of hub proteins in particular. Also, the compelling evidence is accumulated showing that actin cannot

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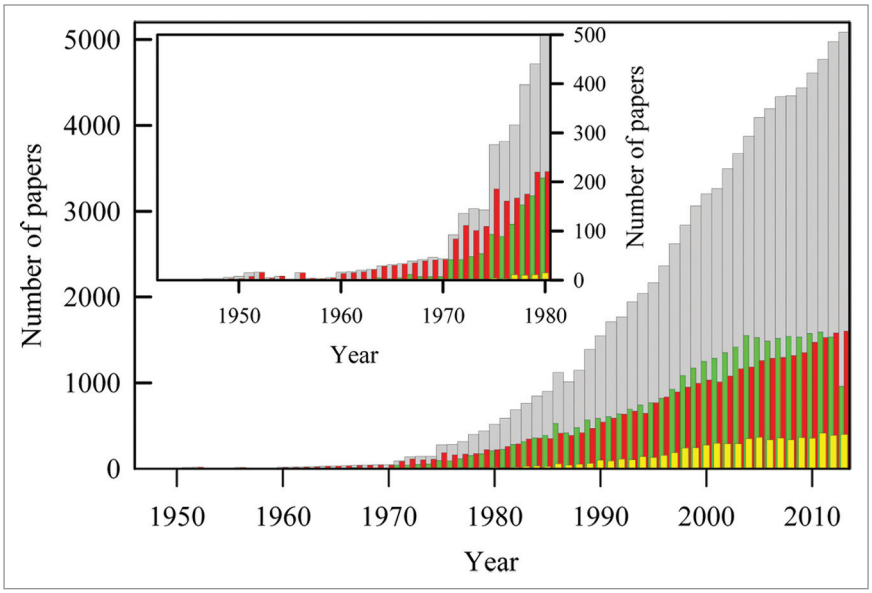

Figure 1. The increase in the interest of researchers toward the actin. The graph illustrates the increase in the total number of publications on actin (gray), with separate tracks showing the number of publications on muscle (red), cytoplasmic (green), and nuclear (yellow) actin from 1950 to the present.

spontaneously fold to the native globular state, and the formation of this state depends on several cellular chaperones. This inability to spontaneously gain ordered structure is also a feature of IDPs. Furthermore, the unique structure of G-actin is known to depend on interaction with metal ions and ATP, which is also typical for IDPs, many of which are able to fold to and maintain ordered state in the presence of specific binding partners. Therefore, actin possesses many of the characteristic structural and functional features of IDPs, such as lack of unique structure in the unbound form, high binding promiscuity, high structural heterogeneity, high susceptibility to various posttranslational modifications, the ability to fold at interaction with specific partners, and the unique capability to fold differently while interacting with different binding partners. On the other hand, one of the functional forms of actin represents a compact globule with the well-defined 3D structure, the fact reflected in the name of this active form, globular actin (G-actin). The goal of this review is to answer the question of whether actin is a typical globular protein or should be classified as intrinsically disordered protein or a hybrid protein possessing both ordered and disordered domains and/or regions. To provide an appropriate background for answering this question, modern views on protein folding should be briefly introduced.

Brief Introduction to the Modern View of Protein Folding

Despite the fact that cell contains numerous factors comprising complex protein folding machinery many proteins can fold spontaneously. In agreement with this observation, one of the truisms in the field of protein folding states: an amino acid sequence of a foldable protein contains a "folding code," using which a polypeptide chain can gain a specific tertiary structure.[18-20] For the first time, this ability of an unfolded protein to gain functional native state was demonstrated in the pioneering experiments conducted by the Anfinsen's group, where the completely unfolded bovine pancreatic ribonuclease with reduced disulfide bridges produced by treatment with mercaptoethanol in 8 M urea was able to fold in native, fully functional state after the removal of urea and mercaptoethanol (e.g., see refs. 21, 22). For these groundbreaking experiments, Christian Anfinsen was awarded the Nobel Prize in Chemistry (1972). Numerous subsequent studies on refolding of small globular proteins supported the validity of this Anfinsen's dogma stating that the unique spatial structure of the native proteins is encoded in its primary structure.[23,24] It is very important to remember though that the coding principles of protein native 3D structure are chiefly different from the coding principles of its amino acid sequence. In fact, protein biosynthesis comprises a set of sequential steps where information encoded in the mRNA nucleotide sequence is step-by-step read by the ribosome and the corresponding amino acids are connected to a polypeptide chain. In other words, the single-dimensional information enclosed in the nucleotide sequence of DNA/RNA is transformed, in a stepwise manner, to the single-dimensional information on the amino acid sequence of a protein. However, such a stepwise mechanism of the information transfer does not work at protein folding, where 3D structure is formed based on the specific contacts between the residues which are distant from each other along the polypeptide chain. Furthermore, the defining role in protein folding is played by some and not all amino acid residues. Because of this, homologous proteins (often with rather low sequence identity) are known to possess similar 3D structure. On the other hand, a single point mutation can dramatically affect the protein folding rate and even completely disturb or even halt correct protein folding.[19,25,26]

The measure of protein stability is free energy, $f = H - TS$, determined by the enthalpy H (i.e., the energy of interaction between the protein's atoms) and the entropy $S = R \ln N$, which is the measure of the number of conformations defining a given state of a protein (R, molar gas constant and T, absolute temperature). The capability of a protein polypeptide chain to fold spontaneously to the specific native state indicates that this native state corresponds to the free energy minimum. However, one should keep in mind that, strictly speaking, any consideration of protein folding should include solvent. Therefore, the native state of a protein corresponds to the free energy minimum of the system including both protein and solvent. This is because of the important role of hydrophobic interactions in stabilization of protein structure. These hydrophobic interactions and the

consequent hydrophobic collapse are determined by the "desire" of protein's nonpolar groups to avoid aqueous environment and result in the "liberation" of water molecules, which otherwise form ordered, ice-like structures around the nonpolar protein groups. Therefore, the formation of the hydrophobic core of a protein macromolecule is accompanied by a noticeable and beneficial decrease in the free energy of the protein-solvent system determined by the increase in the solvent entropy.

Protein folding process can be described as a search for the unique native structure corresponding to the free energy minimum among the astronomically large number of conformations available to the polypeptide chain due to the rotational isomerization around mostly the N-C_α (the dihedral angle φ) and the C_α-C (the dihedral angle ψ) bonds. Since the rigidity and considerable double-bond character of the peptide bond precludes the free rotational isomerization around the C-N bond, for a pair amino acids linked by a peptide bond, six atoms lie in the same plane, the α-carbon atom, and C-O group from the first amino acid and the N-H group, and α-carbon atom from the second amino acid. Despite the fact that two configurations (*cis*- and *trans*-) are possible for a planar peptide bond, almost all peptide bonds in proteins are *trans*. The noticeable exception from this rule is the *trans-cis* isomerization of the peptide bond preceding the proline residue, due to which some peptide bonds in many ordered proteins are in *cis*-configuration. In contrast with the peptide bond, the N-C_α and C_α-C bonds are pure single bonds, and the two adjacent rigid peptide units may rotate about these bonds, taking on various orientations. Since the energy barriers separating configurations of a polypeptide chain corresponding to the free energy minima determined by the rotation around the φ and ψ angles are only about 1 kCal/mol, thereby being relatively close to the thermal energy at room temperature (0.6 kCal/mol), the almost unrestricted rotational isomerization around the N-C_α and the C_α-C bonds allows proteins to fold in many different ways. In fact, assuming that each amino acid in a polypeptide chain can exist, on average, in eight conformations, a small polypeptide chain of 100 residues can adopt up to 8^{100} (or 2×10^{90}) conformations. Therefore, even for such a small protein, spontaneous folding considered as a random sequential search for the native state among the astronomical number of alternative conformations would take astronomical time, longer that the age of the universe.[27] However, a typical small globular protein can find its native state corresponding to the free energy minimum efficiently and fast. In fact, it takes usually less than a second for such a protein to fold. This gives rise to the "Levinthal's paradox": most small proteins fold spontaneously on a millisecond or even microsecond time scale, suggesting that the information on the native state structure and the roadmap on how to reach this structure are both encoded in the protein amino acid sequence.

Since the amino acid sequence contains information on the functional 3D structure of the foldable (ordered) protein, protein folding is often regarded as a realization of the second part of the genetic code. A typical unfolding pathway of a typical globular protein represents a smooth ride that starts with the ordered conformation followed by the formation of various partially folded states with the decreasing amount of residual structure and finally culminates in the appearance of a highly disorganized conformation known as unfolded state.[28] This is illustrated by a simple scheme describing a typical unfolding process:

$$N \rightleftarrows I_1 \rightleftarrows I_2 \rightleftarrows \ldots \rightleftarrows I_M \rightleftarrows U \quad (1)$$

where *N* is a native protein, *U* its unfolded state, and I_1, I_2, and I_M are differently unfolded intermediate states with the decreasing amount of residual structure.

After unfolding, many globular proteins can refold into their native, biologically active structure, suggesting that all the information needed for a given polypeptide chain to fold into a unique tertiary structure is encoded in its amino acid sequence.[22] Furthermore, amino acid sequences must also bear information about pathways defining formation of native structure from the unfolded state. Another important feature of the energy surface describing the conformational behavior of a given foldable protein is the existence of a free energy barrier between the native and/or ordered and denatured states.[19] This circumstance determines the correct function of globular proteins since the existence of a free energy barrier between the ordered and disordered states defines the ability of a globular protein to stay folded and functional.

The unfolded state is entropically favorable because it represents a dynamic ensemble of a large number of conformations that originate from the rotational isomerization of the main chain. In contrast, any compact state imposes significant restrictions on the conformational freedom of the polypeptide chain and is therefore entropically unfavorable. The capability of a given polypeptide chain to attain a compact state is determined by its ability to form intramolecular contacts that compensate for the free energy increase that is caused by the decrease in the entropy component. The compactness of a structure formed by a polypeptide chain is determined by its amino acid composition and sequence. Therefore, depending on the peculiarities of their compositions and sequences, newly synthesized amino acid chains would adopt globular or partially or completely disordered structures.[28]

The structures formed by a polypeptide chain in water are significantly different from that of the Gaussian coil. This difference arises because water is a poor solvent for a polypeptide chain, not only due to the existence of numerous hydrophobic amino acid residues but also because water is a poor solvent for the protein backbone. In fact, despite the absence of hydrophobic residues, polar polypeptides (polyglutamine and glycine-serine block copolypeptides) prefer ensembles of collapsed structures in aqueous milieu.[29,30] Furthermore, residual secondary structure is repeatedly found in unfolded states of foldable globular proteins, even in concentrated solutions of strong denaturants, such as 8 M urea or 6 M guanidine hydrochloride (GdnHCl), which are much better solvents for polypeptide chains than water.[31,32]

Research during past several years clearly indicated that intrinsically disordered proteins (IDPs) or proteins with long intrinsically disordered regions (IDPRs) are very common in nature and are abundantly involved in numerous biological processes.[33-68] In addition to IDPs, there are numerous hybrid proteins that consist of a mixture of ordered and disordered regions. Being biologically active, IDP does not have rigid 3-D structure and exists as a structural ensemble, either at the secondary or tertiary level. To some extent, the conformational behavior and

structural features of some IDPs and IDPRs resemble those of partially folded states of globular proteins, which may exist in at least four different conformations: ordered, molten globule, pre-molten globule, and coil-like.[39,69-71] Using this analogy, IDPs and IDPRs might be classified as native molten globules, where intrinsic disorder is present in the collapsed form, and native coil or native pre-molten globule, where intrinsic disorder is present in the extended form.[39,43,44] More careful analysis of the multitude of structures and/or conformations attainable by the disordered and hybrid proteins suggested that a sequence of an IDP represents a very complex mosaic and typically contains a multitude of elements coding for potentially foldable, partially foldable, differently foldable, or not foldable at all protein segments, and therefore structure of a protein molecule can be considered as a continuous spectrum of differently disordered conformations extending from fully ordered to completely structure-less proteins, with everything in between.[72]

IDPs are known to play diverse roles in regulation of the function of their binding partners and in promotion of the assembly of supra-molecular complexes. The conformational plasticity of IDPs/IDPRs and their intrinsic lack of rigid structure results in a number of exceptional functional advantages, providing them with unique capabilities to act in functional modes not achievable by ordered proteins. Since these advantages were systemized in several recent reviews, only several illustrative examples are given below.[35,40,42,45-50,56,58,60,61] Because sites within their polypeptide chains are highly accessible, IDPs/IDPRs can undergo extensive post-translational modifications, such as phosphorylation, acetylation, ubiquitination, sumoylation, etc., allowing for modulation of their biological function.[42,73]

Many IDPs contain multiple relatively short functional elements. Given the existence of multiple functions in a single disordered protein, and given that each functional element is relatively short, alternative splicing could readily generate a set of protein isoforms with a highly diverse set of regulatory elements.[74] One IDP can bind to multiple partners gaining very different structures.[58] IDPs can form highly stable complexes, or be involved in signaling interactions where they undergo constant "bound-unbound" transitions, thus acting as dynamic and sensitive "on-off" switches. The ability of these proteins to return to the highly flexible conformations after the completion of a particular function, and their predisposition to gain different conformations depending on the environmental peculiarities, are unique physiological properties of IDPs which allow them to exert different functions in different cellular contests according to a specific conformational state.[42]

Mysteries of Actin Structure

According to classical structure-function paradigm, unique protein function is defined by the unique 3-D structure of a protein, which, in its turn, is defined by a unique amino acid sequence.[75] Although the subsequent studies extended the original "lock and key" model of protein action to "induced fit" or "hand-glove" model in order to explain conformational changes associated with protein-ligand interaction and to the subsequent models where the functional ability of a protein was associated with the existence of intramolecular mobility, the validity of this "one sequence-one structure-one function" concept was unquestioned for a long time, especially after the crystal structures of proteins started to be solved by X-ray diffraction. Obviously, this paradigm cannot explain the polyfunctionality of actin (for the description of various functions ascribed to actin, see section "The Mystery of Actin Function"). In this section we will try to understand which structural features make actin so special.

Actin is a highly conserved protein of approximately 42 kDa, and its polypeptide chain consists of 375 amino acids.[76] There are three isoforms of actin that are produced by different genes (α-, β-, and γ-actins), all of which are polymorphic proteins that are capable to polymerize. The actin isoforms differ by only a few amino acids, with most of the variation occurring toward their N-termini.[77]

A very distinctive feature of actin is its ability to polymerize. At low ionic strength in vitro, actin exists as a monomer (G-actin). In the presence of neutral salts, the protein polymerizes to form a single-stranded polymer (the so-called fibrous form of actin, or F-actin). The strong intrinsic propensity of actin to polymerize prevents it from the crystal formation. As a result, no 3D structure is known for the non-complexed protein. However, actin loses its ability to polymerize after forming a complex with some ABPs, and therefore it can be crystallized in the presence of these ABPs, such as DNase I (PDB ID: 1ATN),[78] a *Vibrio parahemolyticus* effector protein VopL (PDB ID: 4M63),[79] chimera of gelsolin domain 1 and C-terminal domain of thymosin β-4 (PDB ID: 1T44),[80] and many other proteins.

The actin monomer in its complex with DNase I (PDB ID: 1ATN)[78] is a relatively flat molecule with the dimensions of 55 × 55 × 35 Å. Actin folds into two major α/β-domains (**Fig. 2A**). Each of these large domains consists of two subdomains, giving rise to a four-subdomain nomenclature that has been traditionally adopted to describe structural features of this protein.[78] Subsequently, actin structures with certain other ABPs were determined. Nonetheless, it was unclear whether the structure of actin in complex with ABPs differs from that of free, native actin. This problem was solved when the structure of actin with a small molecule, tetramethyl-rhodamine-5-maleimide, which prevents actin polymerization, was determined.[81] To date, over 80 structures of actin in complexes with various ABPs have been reported.[82]

Comparison of actin structures available for various forms of the G-actin (modified proteins from different organisms, actin bound to small molecules, or to different nucleotides (ATP or ADP) or even to different ABPs) revealed that the actin monomer possesses very similar structure except to some small but important differences. One of the important structurally variable regions is the so called DNase I loop. This loop includes residues 39–51, which are located at the top of domain 2, and is referred to as the DNase I-binding loop because it is responsible for the formation of the actin complex with DNase I.[78] At the same time, this loop plays a critical role in the inter-subunit contacts in the F-actin filament. Any changes in this loop are known to lead

to the loss of the ability of actin to polymerize. Interestingly, this loop, which forms a β-strand in one crystal structure,[78] was found to be disordered in several other crystal structures[83] and to form an α-helix when ADP, rather than ATP, is bound to actin.[84] In agreement with this statement, **Figure 2B** represents a set of conformations accessible by this loop in different actin structures and shows that, depending on the peculiarities of its environment, a portion of this loop can be found in β-strand, α-helical or irregular structure.

The differences between the ATP- and ADP-bound states are relatively minor and primarily involve two loops: the Ser14 β-hairpin loop, which is located in actin subdomain 1, and the sensor loop carrying the methylated His73.[81] Although the nucleotide-dependent conformational changes in these loops are rather minor, we believe that they are very important and can potentially explain how different ABPs, such as profilin and cofilin, are regulated by the nucleotide bound to actin even though they are not expected to directly contact the nucleotide. In fact, a nucleotide exchange factor profilin has a clear preference for ATP-actin, whereas ADF/cofilin binds ADP-actin with higher affinity than ATP-actin.[82]

On the side opposite to the large cleft of the actin molecule, there is a smaller cleft, which participates in the formation of inter-monomer contacts during actin polymerization, when the loop containing residues 41–45 binds to residues 166–169 and 375.[78] There are two additional contacts between subdomains 3 and 4, where residues 322–325 bind to residues 243–245, and the loop containing residues 286–289 binds to residues 202–204.[85] Since this smaller cleft between domains 1 and 3 also appears to be the binding site for several major ABPs, it is likely that when actin is bound to an ABP it loses the ability to polymerize and can therefore be crystallized. The actin residues that participate in the formation of contacts with ABPs include Tyr143, Ala144, Gly146, Thr148, Gly168, Ile341, Ile345, Leu346, Leu349, Thr351, and Met355 (**Fig. 2A**).[82] Although this cleft is referred to as hydrophobic, not all of the residues mentioned above fit that description. It has been suggested that communication between the two clefts provides the structural basis by which nucleotide-dependent conformation changes modulate the binding affinities of ABPs.[82]

Formation of the fibrillar actin (F-actin) is accompanied by the flattening of a protein structure that moves subdomain 4 toward the helix axes, whereas other subdomains are located at about the same distance from the helix axes (see **Fig. 2C and D**). This structural rearrangement facilitates the intermolecular interactions between the F-actin molecules. Interestingly, the structure of F-actin was determined by X-ray analysis on the basis of the previously determined structure of the G-actin monomer.[86,87] F-actin was shown to form a single helix consisting of 13 molecules repeating in almost exactly six left-handed turns.[86,87] Recently, this helix was directly visualized by electron cryomicroscopy.[88] At the same time, electron microscopy of stained actin fibers showed F-actin to be made of two chains that twist gradually around each other to form a right-handed, two-chained long helix.[82,89,90] Surprisingly, the image of F-actin as two-chained helix appeared to be so impressive that many researchers even today consider F-actin to be a two-chained helix. Nonetheless, this misconception is not inoffensive carelessness, as in this case, the model of the assembly and disassembly of actin

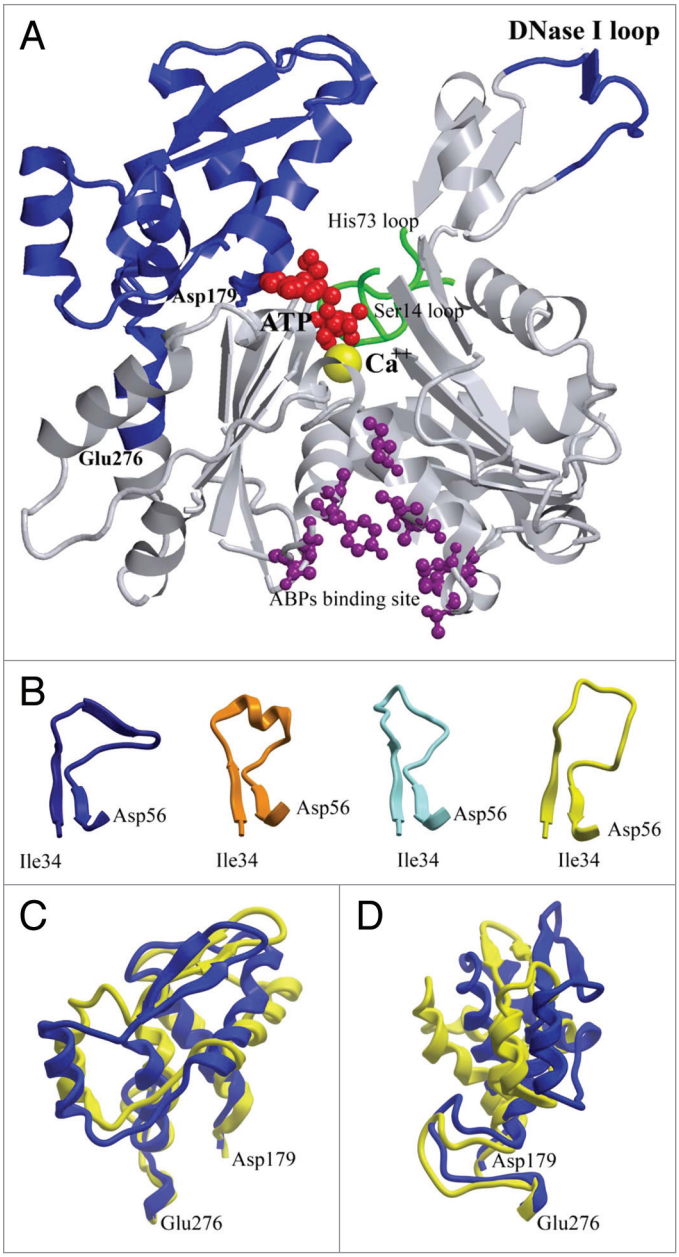

Figure 2. The actin structure. (**A**) Crystal structure of actin monomer. The figure was created on the basis of the PDB data,[180] the file 1ATN,[78] using the graphical software VMD[181] and Raster 3D.[182] ATP (red), Ca^{2+} (yellow), ATP/ADP sensing loops (green), the DNase I loop (blue), and ABPs binding sites are specially emphasized. (**B**) Crystal structure of the DNase I loop of DNase I-actin complex (dark blue, PDB ID: 1ATN[78]), tetramethylrhodamine-5-maleimide-actin complex (orange, PDB ID: 1J6Z),[84] Gelsolin Segment 1 Fused to Cobl Segment-actin complex (cyan, PDB ID: 3TU5),[183] actin monomer of F-actin (blue, PDB ID: 2ZWH).[184] (**C**) Front view of the shift of the residues 179–276 at the G-F-actin transition. DNase I-actin complex (dark blue, PDB ID: 1ATN)[78] and subunit of F-actin (blue, PDB ID: 2ZWH)[184] are superimposed at subdomains 1 and 2. (**D**) Side (left-hand side) view of this shift.

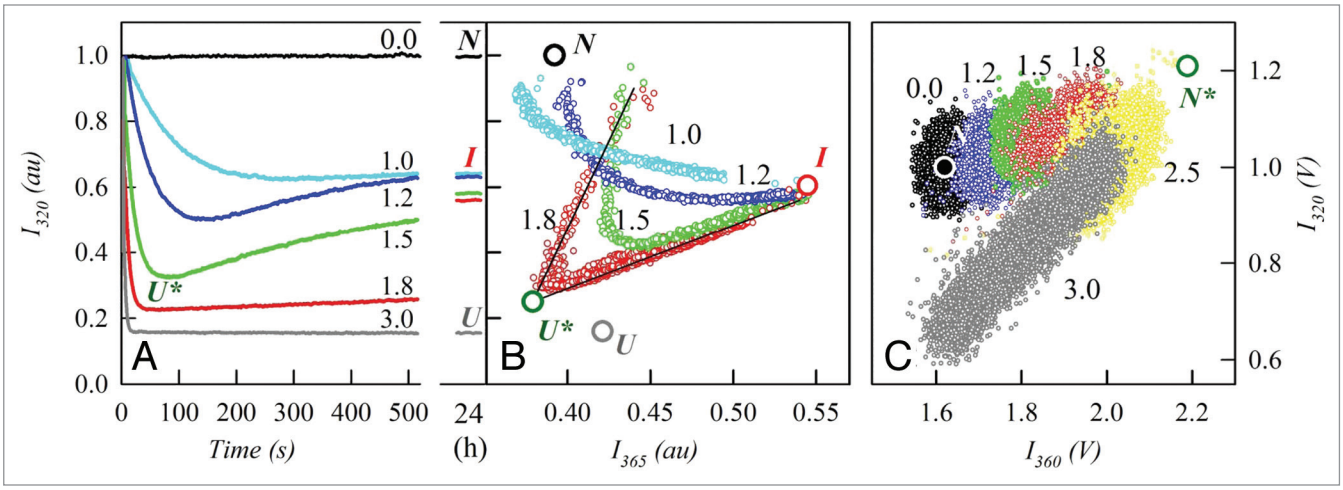

Figure 3. Actin denaturation induced by GdnHCl. (**A**) The kinetics of actin denaturation induced by GdnHCl. The values on the curves indicate the concentration of GdnHCl. (**B**) Parametric dependencies of fluorescence intensity at 320 and at 365 nm; the parameter is the time from the beginning of denaturation. The averaging time of signal is 0.6 s. The values on the curves indicate the concentration of GdnHCl. λ_{ex} = 297 nm. (**C**) Parametric dependencies of fluorescence intensity at 320 and at 360 nm; the parameter is the time from the beginning of denaturation. The averaging time of signal is 0.1 ms. The values on the curves indicate the concentration of GdnHCl. λ_{ex} = 297 nm. The figure (**A**, **B**) is modified from Povarova et al. (2007).[185]

filaments differs in principle differs from the generally accepted model.

The Mystery of Actin Unfolding in Vitro: Unfolding with a Trap

The first investigation of actin folding and unfolding was performed by Lehrer and Kerwar.[2] This study revealed that the removal of calcium ion by the EDTA or EGTA treatment leads to the transformation of G-actin into an inactivated form in which the protein molecule loses its capability to polymerize.[2] Since this species, despite being inactive and unable to polymerize, was not unfolded and preserved significant structure, this form of actin for many years was considered as a specific intermediate state accumulating during the actin unfolding,[2,91-96] and the corresponding form of this protein was termed inactivated actin (I). Later study revealed that inactivated actin may also be obtained by heat denaturation, exposure to moderate urea or GdnHCl concentrations, dialysis with 8 M urea or 6 M GdnHCl, or spontaneously during storage.[97] On the basis of these observations, it was concluded that the inactivated actin (I) can be considered as an on-pathway unfolding intermediate between the native (N) and completely unfolded (U) states:

$$N \rightarrow I \rightleftarrows U \quad (2)$$

All equilibrium experiments appeared to support this model. Here, inactivated actin was characterized by the intrinsic fluorescence spectrum with maximum at wavelength intermediate between the wavelengths of the native and completely unfolded protein,[94] combined with rather rigid microenvironment of tryptophan residues,[98] a considerable increase of the fluorescence anisotropy value reflecting a considerable decrease in the internal mobility of the tryptophan residues in the inactivated actin,[97] and a noticeable distortion of the secondary structure.[98]

A Perrin plot (1/r vs. T/η dependence, where T and η are temperature and viscosity, respectively) showed that inactivated actin was characterized by the independence of 1/r from T/η, suggesting that inactivation is accompanied by the association of partially folded actin molecules in large particles.[99] Later, the hypothesis that the inactivated actin represents a specific aggregate was proven by gel-filtration and sedimentation experiments.[100] Furthermore, based on these analyses it was concluded that inactivated actin represents a supramolecular, monodisperse complex of 14–16 monomers of the partially unfolded actin.[100]

To further clarify the process of actin unfolding, the kinetics of the GdnHCl-induced unfolding of actin was studied.[101,102] The specific shape of the kinetic profiles which possess clear minima in the range of 1.0–2.0 M GdnHCl (see **Fig. 3**) suggested that the transition from the native to the inactivated state occurs via some essentially unfolded intermediate state of actin, giving rise to the intriguing kinetic scheme of actin unfolding:[101,102]

$$N \xrightarrow{k_1} U^* \underset{k_2}{\overset{k_3}{\rightleftarrows}} I \quad (3)$$

where k_i are the rate constants of the corresponding processes and U* is an essentially unfolded kinetic intermediate, the fluorescent properties of which are similar to those of the completely unfolded state but which possesses rather ordered secondary structure. To examine the properties of the newly identified kinetic intermediate U*, the predecessor of inactivated actin, and to elucidate the roles of inactivated actin and its kinetic predecessor in the processes of actin folding and unfolding, a parametric representation of the kinetic dependencies of the tryptophan fluorescence intensity changes recorded at two wavelengths was studied.[103] **Figure 3B** shows that these curves consist of two branches that are most pronounced for a concentration of 1.8 M. One branch corresponds to the N → U* transition and the other to the U* → I transition. This panel also shows that the fluorescence properties of the kinetic intermediate U* differ

from those of completely unfolded actin (U) in 6 M GdnHCl. The existence of one more intermediate states was established in stopped flow experiments (see **Fig. 3C**).[104,105]

Therefore, based on the detailed investigation of actin unfolding and refolding in the presence of GdnHCl a new kinetic model was proposed:[102]

$$N \rightarrow N^* \rightarrow U^* \rightleftarrows I_1 \ldots \xrightarrow[\text{[GdnHCl]<1.8M}]{} I_n \ldots \rightleftarrows I \xrightarrow[\text{[GdnHCl]=0.2M}]{} I_{aggr.} \quad (4)$$

$$U^* \xrightarrow[\text{[GdnHCl]>3.0M}]{} U$$

$I = I_{15}$ at [GdnHCl] = 0M

According to this model, the transition state N* precedes the transformation of native actin into the essentially unfolded state (U*). The formation of this essentially unfolded state (U*) precedes the formation of completely unfolded (U) or inactivated actin (I). In the processes of folding and unfolding, the essentially unfolded state (U*) is an on-pathway intermediate, whereas inactivated actin (I) is an off-pathway associate, the appearance of which competes with the transition to the native state.[102] Irreversible denaturation of actin and stabilization of partially folded protein molecules denatured (inactivated actin) indicates the relevance of this protein to the IDP.

In this scheme, I_{aggr} is aggregates of inactivated actin, resulting from the action of small concentrations of guanidine hydrochloride on the actin. We have found that the dependence of ANS fluorescence on the concentration of GdnHCl in the inactivated actin solutions is the curve with maximum in the narrow range of small concentrations of denaturant (**Fig. 4**). Furthermore, in the same range of GdnHCl concentrations maximum of the parameter A and light scattering (or even precipitation at high protein concentration) was observed. At the same time these characteristics weakly depends on urea concentration up to the concentration when inactivated actin is unfolded.[106] This means that in this narrow range of GdnHCl concentrations inactivated actin forms large aggregates, and that ANS molecules affinity to these aggregates is very high. ANS incorporates into the hydrophobic pockets between the molecules forming aggregates that result in the dramatic increase in its fluorescence intensity. At the addition of GdnHCl to the inactivated actin solution GdnHCl cations bind with the side chain C = O group of the glutamic acids and glutamine, aspartic acid and asparagine amino acid residues of the molecule. The possibility of such interactions has been shown earlier.[107,108] The actin molecule is negatively charged (pI 5.07) at a neutral pH. With an increase in the number of $GdnH^+$ ions bound to inactivated actin, the number of positively-charged groups increases, and at some concentration of GdnHCl (0.2–0.3 M), the initially negatively-charged molecules become neutral, which leads to their aggregation. Upon the further increase in GdnHCl concentration, the number of positively-charged groups on the surface of the protein molecules will exceed the number of negatively-charged groups. Therefore, the conditions will no longer be favorable for aggregation.

Protein aggregation in the solution of low concentration of GdnHCl is especially pronounced for actin, because in this case large supramolecular complexes of inactivated actin[101,102] are involved in aggregation. Aggregates of other proteins in

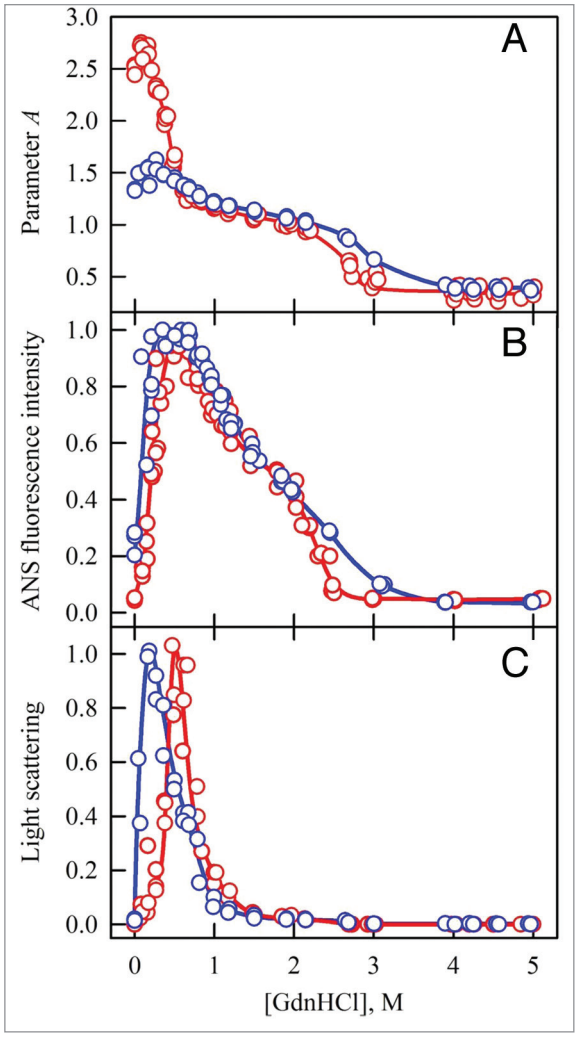

Figure 4. Actin aggregation induced by low concentrations of GdnHCl. The dependence of parameter A (**A**), ANS fluorescence intensity (**B**), and of light scattering (**C**) of the solutions of initially inactivated actin (blue) and initially native actin (red) after 24 h of incubation in a solution of GdnHCl. The protein concentration is 0.15 mg/ml, ANS concentration is 5×10^{-5} M. The figure is modified from Povarova et al. (2010).[106]

intermediate states are comparative small so their formation is not seen by light scattering, but they can be detected using other methods.[106,109,110]

Careful consideration of the unfolding-folding behavior of G-actin is a crucial step for answering the question on whether this protein should be considered as a typical globular protein or should be assigned to a class of intrinsically disordered proteins and hybrid proteins possessing both ordered and disordered domains and/or regions. EDTA-induced calcium removal from actin and action of various denaturing agents (pH, temperature, and chemical denaturants), all result in the formation of the so-called inactivated actin (I-actin), which is a complex compact oligomer containing 14–16 subunits.[100] Since the removal of denaturing agents leads to the formation of I-actin rather than to the complete refolding to the G-actin, denaturation of this protein in vitro is a completely irreversible process. Therefore, I-actin

represents the thermodynamically stable state of a polypeptide chain, information about which is encoded in the amino acid sequence of this protein. Although it is believed that I-actin is functionally inactive, one cannot exclude a possibility that the functional importance of this form of actin is not established as of yet.

As far as G-actin is concerned, this species represents a thermodynamically instable, quasi-stationary form of the protein. This pseudo-stationary state is formed in vivo as a result of complex posttranslational folding events controlled and driven by a set of specific proteins, such as Hsp70 chaperone, prefoldin, and the CCT chaperonin. The G-actin structure is dependent on the ATP and Mg^{2+} binding (which in vitro is typically substituted by Ca^{2+}). Therefore, foldable and "natively folded" forms of actin are always complexes formed by the interaction with the specific protein partners, such as Hsp70 chaperone, prefoldin, and the CCT chaperonin (during the actin folding in vivo) or with Mg^{2+} and ATP (in the native state).

It is likely that the quasi-stationary states of native proteins are rather common in nature. It is likely that the appearance of such states is determined by the intrinsically disordered nature of a given protein, whose polypeptide chain is unable to form ordered compact structure without interaction with its natural partners. In other words, the formation of these quasi-stationary native states represents an energy-intensive process, which is defined by the inability of a given polypeptide to fold spontaneously and which therefore relies on the involvement of specific helpers, such as complex cellular machinery for protein folding, and on interaction with natural partners, such as metal ions, ATP, some other small molecules, etc. How, then, is G-actin formed in vivo? This is a non-trivial question since G-actin represents a thermodynamically instable state which does not correspond to the free energy minimum.

The Mystery of Actin Folding in Vivo

Therefore, a few words are needed about the in vivo folding of this enigmatic protein. Since the conclusion on the reversibility of actin unfolding reported in earlier works[92,95,111,112] was not confirmed in more recent studies,[101,102,104,113] it is likely that the presence chaperones represents an essential requirement for actin folding in vivo.

Chaperones constitute a broad family of proteins with various molecular masses, structures and functions. Two classes of ATP-dependent chaperones, Hsp70 and the chaperonins, are known to play crucial roles in the folding of nascent, non-native polypeptides into their native, functional states inside eukaryotic cells. The Hsp70 chaperones, with the assistance of the co-chaperones of the DnaJ/Hsp40 family, interact with the small hydrophobic clusters of the newly synthesized polypeptide chain.[114] These interactions are not specific because hydrophobic clusters are present in almost every partially folded polypeptide chain. The major role of Hsp70 is likely in preventing undesirable interactions that might result in the aggregation of the newly synthesized polypeptide chain with other molecules. For many proteins, interactions with Hsp70 are sufficient for correct folding. However, the folding of multidomain proteins requires the participation of other helpers. For example, the correct folding of actin relies on its interaction with prefoldin (PFD), which participates in the translocation of the partially folded actin to the CCT chaperonin (chaperonin containing TCP-1; also termed c-cpn or TriC[115-117]).[118] CCT consists of two stacked toroids, each of which contains eight different, albeit homologous subunits. These subunits are multidomain proteins. Their equatorial domains are responsible for the intertoroid interactions and for the interaction with ATP, whereas the apical domains mediate the interaction with the substrate and provide for the passage of the substrate to the central cavity. The folding of actin is a complex, multi-stage, ATP-dependent process controlled by CCT.[119,120] The indispensable participation of PFD and CCT in actin folding is likely the reason that recombinant actin cannot be expressed in *E. coli*,[121] but can be expressed in yeast.[122,123] In agreement with these hypotheses, the refolding of EDTA-denatured actin in the presence of CCT in vitro was observed by Altschuler et al.[105]

Therefore, the amino acid sequence of actin is such that this protein cannot fold into a compact native state without being guided by chaperones. It is important to remember that, despite their crucial roles in the folding of globular proteins in vivo, cellular chaperones do not carry the structural information that is necessary for a newly synthesized polypeptide chain to fold into a unique globular structure. It is very likely that interactions with chaperones and other proteins are even more important for IDPs, preventing them from aggregation and proteolysis.

The Mystery of Actin Function

The exclusive abundance of actin in eukaryotic cells is determined by its multifarious functionality. One of the major, and probably the best studied, function of actin is its ability to polymerize, forming single-stranded polymer (fibrous form of actin, F-actin). In muscle, the F-actin thin filaments and the myosin-based thick filaments are assembled into actomyosin myofibrils, where actin is found within the A and I bands of the sarcomeres. These actomyosin myofibrils are crucial for muscle contraction. Here, myosin heads hydrolyze ATP for energy, which allows them to undergo a cycle during which they attach to thin filaments, exert a tension, and then, depending on the load, perform a power stroke that causes the thin filaments to slide past, shortening the muscle. Another important player in muscle contraction is tropomyosin that is wrapped around the F-actin helix of the helical F-actin filament and covers the active site of actin during the resting phase. This prevents the actin-myosin interaction and thereby prevents muscle contraction.

In addition to its role in muscle cells, actin is an essential component of the cytoskeleton of all eukaryotic cells. The actin cytoskeleton, together with cadherins and catenins, is involved in the cellular adhesion which is a specific characteristic of multicellular organisms leading to tissue specialization and increase complexity.[124] The patterns of gene expression can be

affected by the state of actin polymerization,[125] and in some of the life stages, the F-actin was shown to modify the transcriptomes of some unicellular organisms.[126] Actin plays a role in the cytokinesis leading to the separation of the parent cell into two daughter cells through the constriction of the central circumference in a process that involves a constricting ring composed of actin, myosin, and α-actinin.[127] In apoptosis, cell viability is destroyed in part due to the specific degradation of actin by the ICE/ced-3 family of proteases[128] and calpains.[129] Stress-induced apoptosis also leads to the MAP kinase pathway-controlled reorganization of the actin cytoskeleton and formation of the stress fibers.[130] These processes are regulated by numerous actin-binding proteins (ABPs) that are, in turn, under the control of specific signaling pathways.

Actin is an ATP-ase, nucleotide hydrolysis by actin is a crucial factor regulating the transition between globular and filamentous states. Hydrolysis occurs in the filament. ATP-actin monomers join the fast growing barbed end of the filament, ADP-actin monomers dissociate mainly from the pointed end. The first crucial aspect of polymerization is nucleation, which refers to the formation of a nucleus of three associated monomers and constitutes the rate-limiting phase of polymerization. Actin polymerization is nucleated by the formin proteins, Arp2/Arp3 complex, and its large family of nucleation-promoting factors, Spire, Cobl, TARP, and Lmod.[131] Most actin filament nucleators, with the exception of formins, use WASP homology 2 (WH2) domain for interaction with actin. The WH2 domain has a short length (17–27 amino acids) and is extremely abundant and functionally versatile. Tandem WH2 domains bind three or four actin subunits forming nucleus.

Formins contain two highly conserved formin homology domains, FH1 and FH2, both of which are implicated in control of actin polymerization. It is suggested that the FH2 domain stabilizes actin dimer and this complex is pre-nucleation unit that enables barbed end growth.[132] The proline rich FH1 domain binds to the G-actin binding protein profilin. Profilin deliver ATP-actin monomers to the growing barbed end of actin filament.[132] For a number of filamentous actin structures, nucleation depends on activation of the Arp2/3 complex. For example, Arp2/3 nucleates branched networks that contain short actin filaments found in the lamellipodia of motile cells.[1,133] The Arp2/3 complex consist of seven proteins, including the actin related proteins Arp2 and Arp3, and subunits ARPC1-ARPC5.[134] The complex has low nucleation activity, but it is activated by nucleation promoting factors (NPFs). NPFs are large multidomain proteins capable of catalyzing the formation of a polymerization nucleus, consisting of the two Arps and one to three actin subunits, as well as a conformational change within Arp2/3 complex that allows for monomer addition to the branch and binding of the nucleus to the side of the existing actin filament (mother filament). The new filament (branch) grows at a 70° angle with respect to the barbed end of the mother filament.[132] Then, in the processes of further filament growth, the Arp2/3 complex plays the role of a pointed-end-capping protein that enhances the rapid growth of the filament from its barbed end. The Arp2/3 complex can nucleate filament growth from the side of an existing filament. This ability is important for the dendritic branching that is found at the leading edges of motile cells. It has also been established that certain other proteins that take part in the regulation of filament growth participate in these processes.

The termination of filament growth is regulated by gelsolin and tensin. These proteins bind to the barbed end of the filament and block the addition of new monomers. Gelsolin also is known to participate in the severing of filaments,[135] whereas tropomyosins (a highly conserved family of ABPs) are known to bind along the side of the actin filament to prevent its spontaneous depolymerization and even to protect it from severing by gelsolin. There are several other ABPs that participate in actin filament length determination. These proteins contain domains that allow them to interact with other proteins of the cell signaling networks. This interaction allows the remodeling of the actin cytoskeleton at appropriate times and places within the cell. An example of such an ABP is nebulin, which is an elongated protein with numerous low-affinity actin-binding sites.[136]

When an actin filament is disassembled, F-actin must be depolymerized. The best-characterized proteins that drive depolymerization are the actin depolymerizing factor (ADF) and cofilin family members.[136] After depolymerization, several highly conserved ABPs intervene in the process of actin turnover. These ABPs bind ADP-actin as it is released from the end of the filament (e.g., ADF/cofilin, twinfilin), facilitating the nucleotide exchange from ADP to ATP (e.g., profilin, CapZ actin capping protein) and delivering the actin monomer to the barbed end of a filament to facilitate a new round of polymerization (e.g., profilin, twinfilin, verprolin/WIP, WASP). For rapid filament growth in cells, there must be a sufficiently large amount of ATP-actin ready to polymerize but preserved in the monomer form until an appropriate signal is given. For this purpose, there are special ABPs, the best studied of which are the thymosin family. A special signal triggers the activation of profilin, which leads to the release of thymosin from actin and results in the release of a large amount of ATP-actin that is ready to polymerize.[137] Beyond these examples, F-actin interacts with many ABPs that do not influence its structure and dynamics. These ABPs include myosins that use actin as a track along which to move,[136] cytoskeletal linkers (dystrophin, utrophin, vinculin) that interconnect different cytoskeletal elements and membrane anchors (annexins) that interact with both actin and the membrane.

Furthermore, actin is found not only in cytoplasm, but within the cell nucleus,[14] where it can act as a transcription initiator by interacting with nuclear myosin bound to RNA polymerases and other proteins related to the transcription process.[14] Although actin in the nucleus was discovered at practically the same time as in the cytoplasm,[12] this localization was taken to be an artifact. The focused study on the nuclear actin began only recently. Currently, the presence of actin in the nucleus has been unequivocally demonstrated,[138] together with the crucial roles of actin in regulation of transcription,[15] transcription factor activity,[16] and chromatin remodeling.[17]

However, nuclear actin is less studied than the cytoplasmic form of this protein. The existence of F-actin in the nucleus was controversial for a long time because it was not recognized by phalloidin fluorescence.[139] However, there is a wealth of indirect

evidence that actin in the nucleus must be in a polymerized form. For example, all of the ABPs that interact with F-actin have been detected in the nucleus,[140] and the actin monomer-sequestering drug Latrunculin has been reported to inhibit several nuclear actin-dependent functions, including the export of RNA and proteins,[141] nuclear envelope assembly,[142] transcription,[143] and transcription-induced interchromosomal interaction.[144] The most convincing work in this direction is a recent microscopy study revealing that approximately 20% of the total nuclear actin pool is in the polymeric state.[143] The earlier failure to stain nuclear actin with phalloidin can be explained by the low actin concentrations in nucleus which contains ~1% of cellular actin, the decoration of F-actin by ABPs (such as ADF/cofilin[145]) and possibly by a shorter length.[138] At the same time, the dendritic actin branches have not been visualized in the nucleus, although Arp2/3 and other components that nucleate these filaments were found in the nucleus. The other unsolved problem is the actin transport in and out of the nucleus. There is some evidence that actin can cross the nuclear pore complexes in a complex with profilin and exportin-6, although its import mechanism is still unclear.[138]

Interestingly, the "functional" form of actin differs in the muscle, the cytoplasm and the nucleus. In the muscle, once they are generated, filaments are not disassembled and new filaments appear only during muscle growth or reparation; therefore, the main functional form is F-actin. In non-muscle cytoplasm, although the cytoskeleton is composed of actin fibrils, it can be assembled and disassembled. Cell motility is also based on actin filament polymerization and depolymerization. Therefore, a sufficiently large amount of actin monomers must be stored in the cytoplasm to support the effective function of actin. In the nucleus, for the first time, actin monomers play a significant role by regulating SFR (serum response factor) activity. The actin monomer pool is involved in controlling the expression of many proteins that are themselves components of the actin cytoskeleton.[15]

Based on these considerations it is clear that actin is a multifunctional protein involved in a wide range of interactions with various partners. This statement is further illustrated by **Figure 5A** representing the results of the analysis of interactability of human α-actin using the STRING database,[146] which acts as a "one-stop shop" for all information on functional links between proteins, and version 9.0 of STRING (accessible at http://string-db.org) covers more than 1100 completely sequenced organisms, including *Homo sapience*. **Figure 5A** clearly shows that human α-actin is involved in multiple protein-protein interactions and therefore can be considered as a hub protein. For comparison, **Figure 5** represents the results of similar analysis of a typical ordered protein, hen egg white lysozyme (**Fig. 5B**), and a typical hybrid protein with well-established disorder-based interactome, human p53 (**Fig. 5C**). Clearly, by its binding promiscuity, actin is closer to p53 than to lysozyme.

Enigmatic Relation between Actin Structure and Functions: Intrinsic Disorder to the Rescue

Curiously, **Figure 6A** shows that actin preserve significant mobility even when being complexed with an ABP and crystallized. This is evident from the presence of regions with relatively high values of the B-factor (or the Debye-Waller factor). The B-factor can be taken as indicating the relative vibrational motion of different parts of the structure, where atoms and/or backbone with low B-factors belong to a part of the structure that is well-ordered, whereas atoms and/or backbone with large B-factors generally belong to part of the structure that is very flexible. Furthermore, in its complex with the chimera of gelsolin domain 1 and C-terminal domain of thymosin β-4 (PDB ID: 1T44),[80] actin has two regions of missing electron density (residues 25–27 and 39–50) corresponding to the highly mobile segments, which are likely to be disordered.

The per-residue intrinsic disorder propensity of human actin evaluated by several commonly used disorder predictors is shown in **Figure 6B**, which suggests that this protein belongs to the class of hybrid proteins containing both ordered and intrinsically disordered regions. These disorder propensities were evaluated using the members of the PONDR family of intrinsic disorder predictors. Here, scores above 0.5 correspond to disordered residues and/or regions. PONDR® VSL2B is one of the most accurate stand-alone disorder predictors,[147] PONDR® VL3 possesses high accuracy in finding long IDPRs,[148] PONDR® VLXT is not the most accurate predictor but has high sensitivity to local sequence peculiarities that are often associated with disorder-based interaction sites,[44] whereas PONDR-FIT represents a metapredictor that, being moderately more accurate than each of the component predictors, is one of the most accurate disorder predictors.[149] **Figure 6B** shows that actin has several disordered and flexible regions (e.g., regions with predicted disorder scores exceeding 0.5 or close to 0.5). Some of these regions are residues 1–6, 43–56, 98–115, 140–170, 201–208, 228–249, 300–317, and 364–375. Curiously, some of these predicted disordered and/or flexible regions coincide or overlap with important functional

Figure 5 (See opposite page). Functional characterization of the human α-actin (UniProt ID: P68133) by STRING database (**A**), which is the online database resource Search Tool for the Retrieval of Interacting Genes providing both experimental and predicted interaction information.[146] STRING produces the network of predicted associations for a particular group of proteins. The network nodes are proteins. The edges represent the predicted functional associations. The edges represent the predicted functional associations. An edge may be drawn with up to 7 differently colored lines; these lines represent the existence of the seven types of evidence used in predicting the associations. A red line indicates the presence of fusion evidence; a green line, neighborhood evidence; a blue line, co-occurrence evidence; a purple line, experimental evidence; a yellow line, text mining evidence; a light blue line, database evidence; a black line, co-expression evidence.[146] Here, 240 partners of actin are shown, which were obtained by STRING using the medium confidence level (score above 0.4) and a set of active prediction methods for all the types of evidence: neighborhood, gene fusion, co-occurrence, co-expression, experiments, databases, and textmining. This figure also contains the results of STRINGing of a typical ordered protein, hen egg white lysozyme (**B**, UniProt ID: P00698), and a typical hybrid protein with well-established disorder-based interactome, human p53 (**C**, UniProt ID: P04637). Clearly, by its binding promiscuity, actin is closer to p53 than to lysozyme.

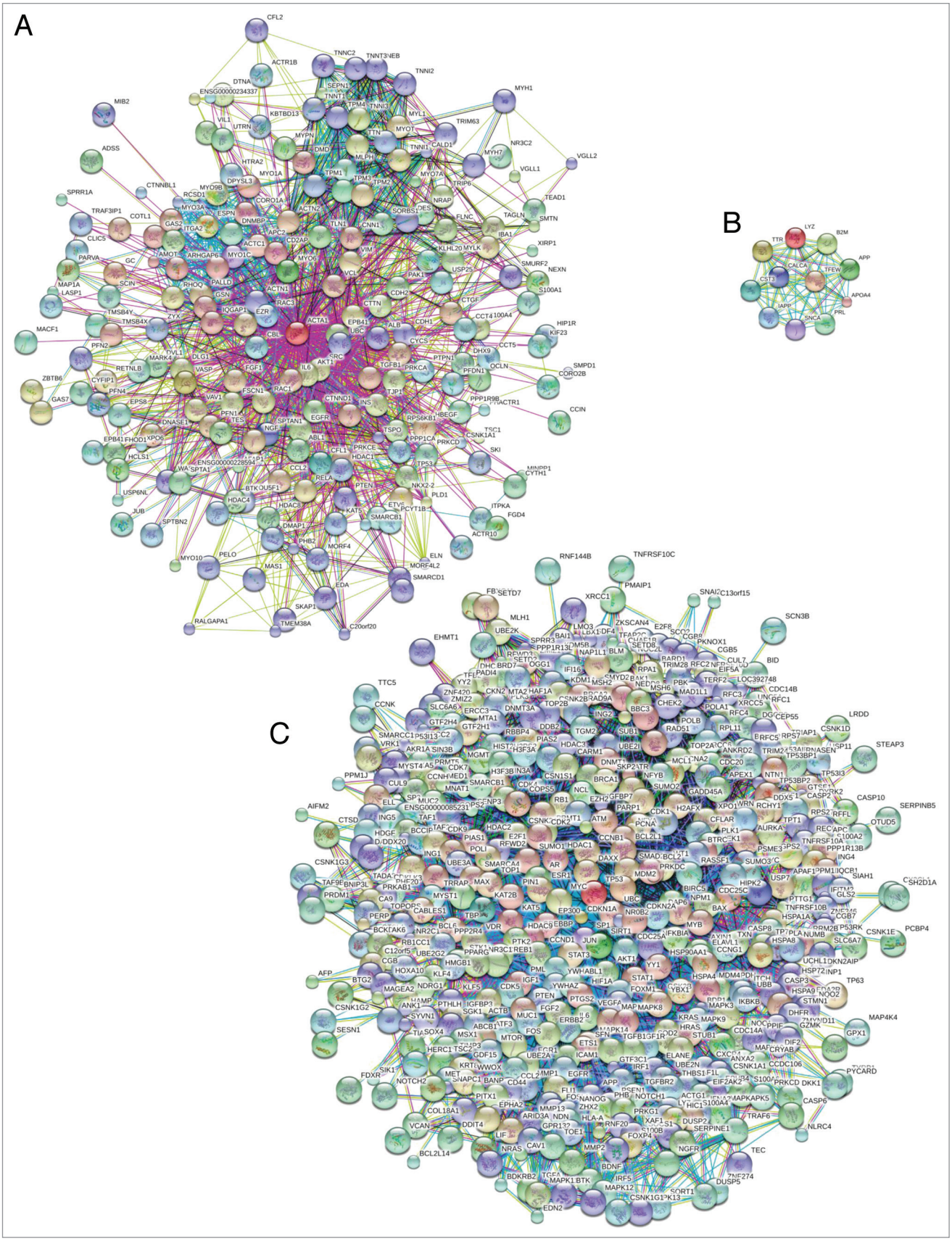

Figure 5. For figure legend, see page 10.

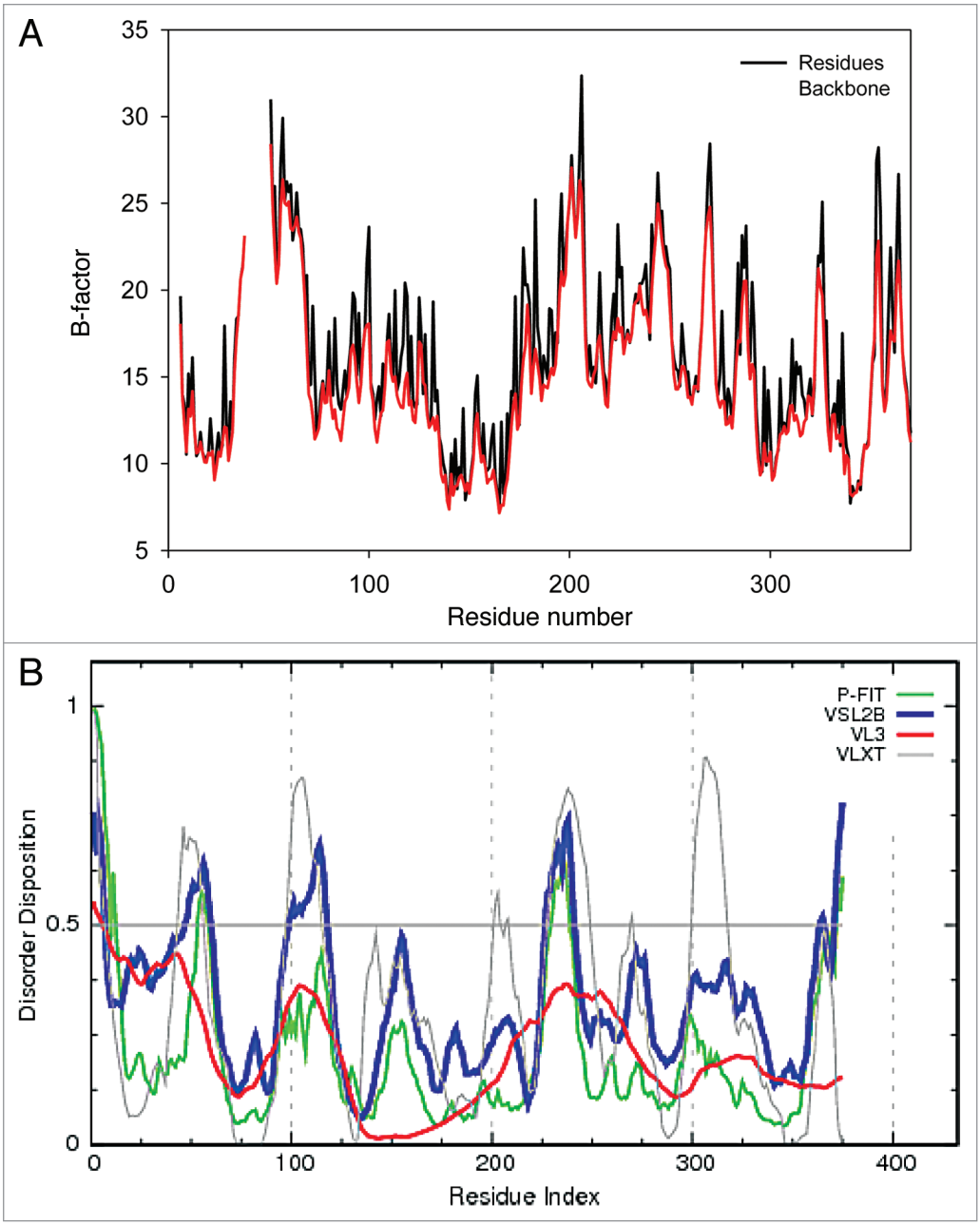

Figure 6. The intrinsic disorder in the actin molecule. (**A**) The B-factor distributions for residues (black curve) and backbone (red curve) in the crystal structure of human actin complexed with the chimera of gelsolin domain 1 and C-terminal domain of thymosin β-4 (PDB ID: 1T44).[80] (**B**) Evaluating intrinsic disorder in human actin by a family of PONDR predictors. Here, scores above 0.5 correspond to disordered residues and/or regions. PONDR® VSL2B is one of the most accurate stand-alone disorder predictors,[147] PONDR® VL3 possesses high accuracy in finding long IDPRs,[148] PONDR® VLXT is not the most accurate predictor but has high sensitivity to local sequence peculiarities that are often associated with disorder-based interaction sites,[44] whereas PONDR-FIT represents a metapredictor that, being moderately more accurate than each of the component predictors, is one of the most accurate disorder predictors.[149]

segments of actin discussed above, such as DNase I loop (residues 39–51), residues involved in interaction with ABPs, and regions responsible for actin polymerization (residues 41–45, 166–169, and 375). In other words, similar to other disordered and hybrid proteins actin possess disordered and/or flexible regions crucial for its function.

Figure 7 provides further evidence for the abundance and significance of intrinsic disorder in human α-, β-, and γ-actins (UniProt ID: P68133, P60709, and P63621). Here, the outputs of the D^{2}P^2 database (http://d2p2.pro/)[150] for these three proteins are shown. Disorder analysis is enhanced by including results of 9 disorder predictors, which generally agree on the existence of at least 5 disordered regions. Importantly, this analysis revealed the existence of multiple posttranslational modification (PTM) sites. The most abundant PTM type is phosphorylation followed by ubiquitination and acetylation. Majority of the PTM sites are located within (on in the close proximity to) the disordered and/or flexible regions. Based on these data, it is clear that actins are hybrid proteins with several biologically important disordered regions.

Among various functional advantages ascribed to IDPs/IDPRs is their ability to be involved in multiple interactions with non-related partners. In fact, IDPs and IDPRs are characterized by exceptional binding promiscuity, where one protein or regions is able to bind to multiple partners.[151] Obviously, the classical molecular recognition mechanisms cannot explain the ability of IDPs/IDPRs to bind to multiple partners.[152] Furthermore, some IDPs/IDPRs were shown to adopt different structures upon binding to different partners,[153-159] thereby serving as conformational switches that play a number of crucial roles in mediating protein-protein interactions (PPIs).[151,153-172] Curiously, one of the disordered segments of actin (namely its DNase I loop, residues 39–51), was shown to be such a conformational switch (see above).

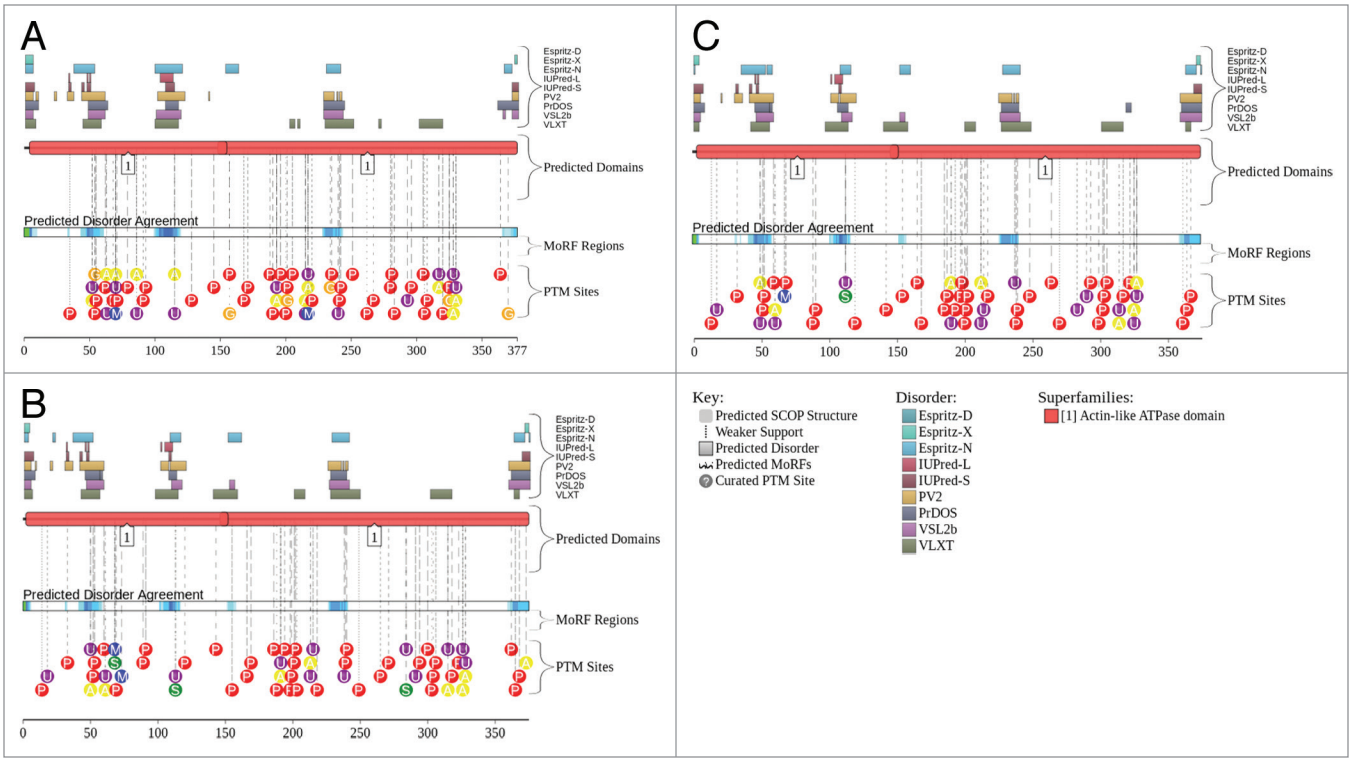

Figure 7. Abundance and functionality of intrinsic disorder in human α- (**A**), β- (**B**), and γ-actins (**C**), UniProt IDs: P68133, P60709, and P63621, respectively. Here, the outputs of the D^{2}P^2 database (http://d2p2.pro/)[150] for these three proteins are shown. Disorder analysis is enhanced by including results of 9 disorder predictors, which generally agree on the existence of at least 5 disordered regions.

In PPI networks, there are several multitasking proteins (known as hubs) that have multiple links. With respect to temporal structure of the PPI networks, some proteins have multiple simultaneous interactions ("party hubs"), while others have multiple sequential interactions ("date hubs").[173] From a functional perspective, "date hubs" may connect biological modules to each other,[174] whereas "party hubs" may form scaffolds that enable the assembly of functional modules.[173] Involvement of intrinsic disorder is one of the reasonable mechanisms for the description of the promiscuity of hub proteins,[151,160-164] where, intrinsic disorder and related disorder-to-order transitions could enable one protein to interact with multiple partners (one-to-many signaling) or to enable multiple partners to bind to one protein (many-to-one signaling).[159] In line with these considerations, intrinsically disordered nature of actin provides a plausible explanation for its hubness.

The potential for partially or completely disordered proteins to form complexes with their partners is the molecular basis of numerous crucial functions of IDPs in signaling, recognition, and the regulation of different intercellular process. Although many proteins are involved in such processes, special attention has been paid to the main regulatory proteins, which play key roles in the regulation of these complex processes. Many of these proteins, which are known as hubs and network concentrators and serve as "conductors" of these biological processes, were shown to be disordered.[58,175] Among such disordered hub proteins are α-synuclein, p53, HMG proteins, estrogen receptor α, and many others.[176-178] Proteome-wide analyses revealed that IDPs and hybrid proteins possessing ordered and disordered regions are more common in eukaryotes than in prokaryotes or archaea, likely due to the more complex regulation and signaling systems in higher organisms.[179] A striking example of this trend is p53, the function of which is inherent only in multicellular organisms. This protein monitors and coordinates practically all of the intercellular processes,[178] prioritizing the organism's needs over the interests of different cells: a damaged cell must either accelerate the repair processes or lose the possibility of division, and it may even die as a result of apoptosis.[178]

Concluding Remarks: Intrinsic Disorder and Quasi-Stationarity as Tools to Solve Actin Mysteries

Actin is a ubiquitous and multifunctional protein. It is one of the main components of the system of muscle contraction; it forms the cytoskeleton; and it is found in the cell nucleus in which, except for the motility and scaffold functions, actin acts as a regulator protein that participates in the processes of transcription and chromatin remodeling.

Based on the data presented in this review, it is evident that actin meets the majority of the characteristics of IDPs or hybrid proteins. In fact, like some other IDPs, actin cannot fold into a compact state without chaperones. This protein not only cannot fold without chaperones but also cannot maintain

a compact structure without its ligands, the metal ions (Ca^{2+} or Mg^{2+}) and ATP. In fact, actin always exists in complexes: while folding, it successively interacts with the chaperone Hsp 70, then with prefoldin (PFD) and finally with the chaperonin CCT, which provides means for correct folding and metal and ATP incorporation; fibrillar actin is formed by the self-association of G-actin molecules; in the cytoplasm or nucleus, actin is in complex with ABPs; and, in particular, the G-actin pool is preserved in complex with profilin; inactivated actin is also a monodisperse complex (not an amorphous aggregate) that, possibly, has some functional role. As many other IDPs or hybrid proteins, actin interacts with an enormous number of partners[28] and possesses numerous PTM sites. While interacting with numerous ABPs, actin acts as a hub protein, as is typical for IDPs and hybrid proteins. Many of the ABPs themselves are IDPs involved in various signaling system and interacting with other hub proteins.

On the other hand, actin is noticeably different from typical IDPs since in its apo-form this protein cannot maintain monomeric state. Being stripped of its binding partners actin is not just loses its unique 3D structure but is almost instantaneously converted to the homogenous oligomeric form, I-actin, which can be considered as an oligomeric molten globule-like state. Furthermore, unfolding of actin is an irreversible process, with I-actin being a final state of the spontaneous refolding in vitro. This indicates that G-actin is a quasi-stationary state, that the information encoded in the amino acid sequence of actin is not sufficient to ensure normal folding of this protein to the globular functional state with the unique 3D structure, and that instead, I-actin, which is formed as a result of the spontaneous refolding of actin in vitro, represents the thermodynamically stable state, information about which is encoded in the amino acid sequence of actin.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

Acknowledgments

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