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ARTICLE *in* CYTOSKELETON · OCTOBER 2013

Impact Factor: 3.12 · DOI: 10.1002/cm.21118 · Source: PubMed

REVIEW ARTICLE
Cytoskeleton, June 2013 00:00–00 (doi: 10.1002/cm.21118)

Intrinsic Structural Disorder in Cytoskeletal Proteins

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Received 11 March 2013; Revised 26 May 2013; Accepted 29 May 2013
Monitoring Editor: Miklós Nyitrai

Cytoskeleton, the internal scaffold of the cell, displays an exceptional combination of stability and dynamics. It is composed of three major filamentous networks, microfilaments (actin filaments), intermediate filaments (neurofilaments), and microtubules. Together, they ensure the physical and structural stability of the cell, whereby also mediating its large-scale structural rearrangements, motility, stress response, division, and internal transport. All three cytoskeletal systems are built upon the same basic design: they have a central repetitive scaffold assembled from folded building elements, surrounded and regulated by accessory regions/proteins that regulate its formation and mediate its countless interactions with its environment, serving to send regulatory signals to and from the cytoskeleton. Here, we elaborate on the idea that the opposing features of stability and dynamics are also manifest in the dichotomy of the structural status of its components, the core being highly structured and the accessory proteins/regions being highly disordered, and are responsible for most of the regulatory (post-translational) input promoting adaptive responses and providing dynamics necessary for each of the cytoskeletal systems. This pattern entails special consequences, in which the manifold functional advantages of structural disorder, most pronounced in regulatory and signaling functions, are all exploited by nature. © 2013 Wiley Periodicals, Inc.

Key Words: protein disorder; unstructured protein; entropic chain; induced folding

Introduction

The cytoskeleton is composed of three basic components: microfilaments (actin), intermediate filaments [neurofilaments (NFs) in neuronal cells], and microtubules (MTs), and it provides the internal scaffold (skeleton) of the cell. It can be considered as a very special organelle, which represents a unique combination of stability and dynamics, physical rigidity and flexibility, long-time persistence and rapid, cataclysmic rearrangements. By providing a special microenvironment, the cytoskeleton ensures the physical separation of cellular constituents, thus segregating and directing cellular activities. It bridges molecular (nano-m) and cellular (micro-m) distances and represent the tracks of transport of cellular constituents over large distances. It provides the locomotive force of cell migration, it drives clustering of membrane proteins, drives cell division and the formation of protrusions the cell uses for exploring its environment. Apparently it does it by a combination of a physically rigid but inherently unstable central scaffold and a flexible and rather variable outer layer of accessory proteins/regions. Due to its central importance in cell physiology, the cytoskeleton is involved in many diseases, ranging from cancer to neurodegeneration [Pajkos et al., 2012; Raychaudhuri et al., 2009; Uversky et al., 2008]. Our central theme here is that multifaceted and highly dynamic behavior is enabled by structural disorder in all three major cytoskeletal constituents, also reflecting their increasing complexity from NFs to the actin cytoskeleton (Supporting Information Table S1, Table I). Intermediate filaments (IFs) have three principal components, IF-L(ight), IF-M(edium), and IF-H(igh), all three of which form an extended coiled-coil structures, from which their variable disordered tails project away [Fuchs and Weber, 1994; Fuchs and Cleveland, 1998]. MTs are hollow tubes of protofilaments, made up of virtual filaments of polymerized tubulin α/β heterodimers. Their stability and interactions with their environment depend on the presence and association of fully disordered accessory proteins, such as microtubule-associated protein 2 (MAP2), tau protein, and stathmin [Alexa et al., 2002; Cassimeris, 2002; Dehmelt and Halpain, 2005]. The most diverse and versatile component of the cytoskeleton is microfilaments, which contain filamentous actin (F-actin) regulated in diverse ways by largely disordered accessory/regulatory proteins (e.g., Tβ4 and Wiskott–Aldrich syndrome protein [WASP]).

Additional Supporting Information may be found in the online version of this article.

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Table I. Select Examples of Cytoskeletal Proteins

Protein*	Uniprot	Length	Predicted disordered residues	Ratio-disordered residues	Average LDR* length	Role*
Intermediate filament-associated proteins						
Vimentin	P08670	466	121	0.26	32.0	Type III intermediate filament protein, providing the major cytoskeletal element in mesenchymal cells
Plectin	Q15149	4684	1303	0.28	70.9	Linker between microfilaments, microtubules, and intermediate filaments, also links the cytoskeleton to plasma membrane junctions
Filaggrin	P20930	4061	3913	0.96	3886	Crosslinking keratin fibers with other cytoskeletal elements in epithelial cells
Keratin	Q8N1N4	1317	941	0.72	52.3	Forms IF bundles in the outer layer of human skin, hair, and nails
Lamin	Q5TCI9	513	321	0.63	40.1	(Nuclear) lamins (Class V IF proteins) interact with membrane proteins to build up nuclear lamina in the nucleus
Microtubule-associated proteins						
Tau	P10636	758	714	0.94	335.5	Promotes microtubule assembly and stability
MAP-2	P11137	1827	1591	0.87	172.9	Stabilizes microtubules against depolymerization
MAP-1A	P78559	2803	2371	0.84	787.3	Structural protein involved in the filamentous cross-bridging between microtubules and other skeletal elements
Stathmin	P16949	149	138	0.92	131	Destabilizes microtubules: prevents their assembly and promotes their disassembly
CLIP1	P30622	1438	825	0.57	98.3	Binds to the plus end of microtubules and promotes microtubule growth and bundling. Also links cytoplasmic vesicles to microtubules
Dynactin subunit 1 (p150glued)	Q14203	1278	619	0.48	109.3	Involved in dynein-driven retrograde movement of vesicles and organelles along microtubules
APC	P25054	2843	2155	0.75	148.4	Mediates ERBB2-dependent stabilization of microtubules at the cell cortex
MACF1	Q9UPN3	7388	1557	0.21	61.1	Cross-links microtubules with actin filaments. Plays an important role in ERBB2-dependent stabilization of microtubules at the cell cortex
Kinesin-1 heavy chain	P33176	963	376	0.39	60.5	Microtubule-dependent motor required for normal distribution of mitochondria and lysosomes
EB1	Q15691	268	68	0.25	45	Binds to the plus end of microtubules, and regulates microtubules dynamics. May be involved in spindle function by anchoring microtubules to the centrosome

TABLE I. *Continued*

Protein*	Uniprot	Length	Predicted disordered residues	Ratio-disordered residues	Average LDR* length	Role*
Actin-associated proteins						
WASP	P42768	502	382	0.76	180	NPF regulating actin filament reorganization via interaction with Arp2/3 complex
Cordon-bleu	O75128	1261	1030	0.82	163.5	NPF controlling neuronal morphology, especially at sites of high actin dynamics
Spire	Q08AE8	756	306	0.41	62.8	NPF of nonbundled, unbranched actin filaments, involved in vesicle transport
Cortactin (EMS1)	Q14247	550	341	0.62	147	Organizes actin cytoskeleton in cell structure, lamellipodia/ invadopodia formation, highly expressed in tumor cells
SCAR/WAVE	Q92558	559	387	0.69	330	NPF involved in signaling from receptors to the actin cytoskeleton
Thymosin beta (B4)	P62328	44	44	1.00	44	G-actin-sequestering protein that interacts with F-actin and regulates actin-driven assembly
Supervillin	O95425	2214	1213	0.55	200.8	Links the actin cytoskeleton with the cellular membrane and signaling pathways, modulates the formation of focal adhesions and lamellipodia/invadopodia
Juxtanodin (or Ermin)	Q8TAM6	284	261	0.92	251	Organizes actin cytoskeleton in central nervous system cells, mainly in oligodendrocytes
JMY	Q8N9B5	988	466	0.47	109	NPF in the cytoplasm, whereas in the nucleus a transcription coactivator that binds p300
Epsin	Q9Y6I3	576	448	0.78	435	Actin-bundling protein that regulates receptor-mediated endocytosis, and regulates membrane curvature

About 25 examples of proteins associated with the three major cytoskeletal components (intermediate filament, microtubules, and actin filaments). The proteins are the ones discussed in the text, with characteristic features of their predicted disorder (total length, number of disordered residues, the ratio of their disordered residues, the number and average length of their long disordered regions).

*abbreviations: APC: Adenomatous polyposis coli; Arp2/3: Actin-regulatory protein 2/3; CLIP1: CAP-GLY domain containing linker protein 1; EMS1: Cortactin; ERBB2: Human epidermal growth factor receptor 2 (also known as HER2); IDR: Intrinsically disordered region; JMY: Junction-mediating and -regulatory protein; MACF1: Microtubule-actin cross-linking factor 1; MAP2 (1A): microtubule-associated protein 2 (1A); NPF: nucleation-promoting factor; SCAR/WAVE: suppressor of cAR/WASP family Verprolin-homologous protein; WASP: Wiskott–Aldrich syndrome protein.

The likely importance of structural disorder in all three systems results from the special functional modes it permits. For many proteins, termed intrinsically disordered proteins/regions (IDP/IDR), the entire protein or its segment lacks a well-defined tertiary structure, rather it exists in an unfolded state with no tertiary and only transient secondary structural contacts. This dynamic structural ensemble is maintained by the highly hydrophilic nature of their polypeptide chain [Uversky et al., 2000]. The most comprehensive repository of IDPs/IDRs, the DisProt database [Sickmeier et al., 2007], holds about 1500 disordered regions within about 700 proteins. Structural disorder is typically higher in eukaryotes (5–15% of proteins are fully disordered and about 50% have at least one long disordered region) than in prokaryotes [Burra et al., 2010; Pancsa and Tompa, 2012]. Structural disorder abounds in functional categories associated with signal transduction, regulation of transcription, and chromatin organization [Tompa and Csermely, 2004; Ward et al., 2004; Xie et al., 2007]. There are two basic modes of action of IDPs/IDRs, their function either stems directly from their disorder (entropic chains, e.g., linkers, entropic bristles, etc.) or from molecular recognition/interaction (e.g., binding their partner via short recognition elements [Davey et al., 2006; Diella et al., 2008] or disordered domains [Tompa et al., 2009] in a process of induced folding [Wright and Dyson, 2009]). The functional outcome in both types of functions is different from the action of folded proteins. Entropic chain functional modes are not accessible to folded proteins, whereas in recognition functions structural disorder may uncouple specificity from binding strength, enable adaptability to different binding partners [Davey et al., 2011; Huang and Liu, 2013; Tompa et al., 2005] often effectively regulated by post-translational modifications [Iakoucheva et al., 2004], and mediate interactions with multiple partners as hubs in protein–protein interaction networks [Dosztanyi et al., 2006; Hegyi et al., 2007].

In a sense, the field of structural disorder is still in its infancy, and much work is needed to bring it to the descriptive and predictive level of classical structural biology, so as to deserve the term "unstructural" biology [Tompa, 2011]. The characterization of structural disorder is usually achieved by two complementary approaches. Bioinformatics predictions of structural disorder is now based on a variety of principles, such as amino acid propensity [Prilusky et al., 2005; Uversky et al., 2000], secondary structure preference [Liu and Rost, 2003], contact potentials of amino acids [Dosztanyi et al., 2005; Schlessinger et al., 2007], or more complex relationships between sequence and disorder, captured by machine learning algorithms [Peng et al., 2005] or meta-approaches [Ishida and Kinoshita, 2008; Schlessinger et al., 2009]. The ever-increasing accuracy and dependability [Monastyrskyy et al., 2011] of these approaches positioned bioinformatics in the center of addressing questions at the genome/proteome level, such as the phylogenetic distribution of disorder [Pancsa and Tompa, 2012; Xue et al., 2010], its correlation with different functional categories [Ward et al., 2004] and involvement in disease [Hegyi et al., 2009; Iakoucheva et al., 2002; Pajkos et al., 2012]. Bioinformatics can also outline functional elements in individual disordered proteins, such as short binding motifs [Davey et al., 2006; Fuxreiter et al., 2007], post-translational modification sites [Iakoucheva et al., 2004], and sites of protein–protein interactions [Dosztanyi et al., 2009].

Detailed structural–functional insight on disorder, however, can only be expected from powerful biophysical methods. Collectively, they have provided evidence that IDPs are not featureless (random coil-like) polypeptide chains, they have diverse, function-related, transient short-and long range structural organization. The uncontested champion of IDPs is nuclear magnetic resonance (NMR), which provides residue-level data on structural preferences and dynamic features of proteins in the disordered state. The technique can be complemented by a range of other approaches, such as small-angle X-ray scattering (SAXS), circular dichroism (CD), calorimetry (isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC)), fluorescence spectroscopy, X-ray crystallography, and many more. The combination of distinct biophysical approaches and advanced computational tools enables to describe the real ensemble of IDP/IDR structures [Fisher and Stultz, 2011]. In the case of cytoskeletal proteins, such ensemble description has been achieved for tau protein [Mukrasch et al., 2009] (cf. Fig. 3). A descriptive list of human cytoskeletal proteins (from the DisProt database [Sickmeier et al., 2007]) for which there is biophysical evidence and characterization of the involvement of disorder, is provided in Supporting Information Table S2.

In this review, we would like to describe the great variety of structural/functional associations of structural disorder in the cytoskeleton. Bioinformatics predictions [Ward et al., 2004] and scattered experimental observations [Czisch et al., 1993; Hernandez et al., 1986; Mukrasch et al., 2009] already provided evidence for the frequent and important involvement of structural disorder in the organization and regulation of cytoskeleton. As already suggested, all three major constituents of the cytoskeleton have similar basic design: they have a central fibrillar core made of structured building blocks (coiled-coil head-domain in NFs, G-actin in microfilaments and tubulin heterodimers in MTs), regulated by a great variety of accessory proteins (side-arms in the case of neurofilaments), which carry out diverse functions and usually show a high level of structural disorder (cf. Fig. 1 through 5). Altogether, our search for cytoskeletal proteins in UniProt resulted in 1457 unique hits for *Homo sapiens* (Supporting Information Table S1, see also Table I for select examples), which overall show a high level of structural disorder (28%). The number of components associated with the three cytoskeletal systems in humans (IFs: 160, MTs: 358, actin filament: 1029) clearly show

their increasing complexity, perhaps not accidentally also correlating with their average disorder (IFs: 0.13, MTs: 0.31, actin filament: 0.30). The distribution of structural disorder shows substantial heterogeneity (Fig. 1A, many proteins with little disorder, mostly core components and modifying enzymes, and many with higher disorder, mostly other regulatory proteins cf. Figs. 1B and 1C). On the average, cytoskeletal proteins have two long IDRs (Fig. 1C). In all, more than 40% of cytoskeletal proteins have more than 30% of their residues disordered (cf. Supporting Information Table S1).

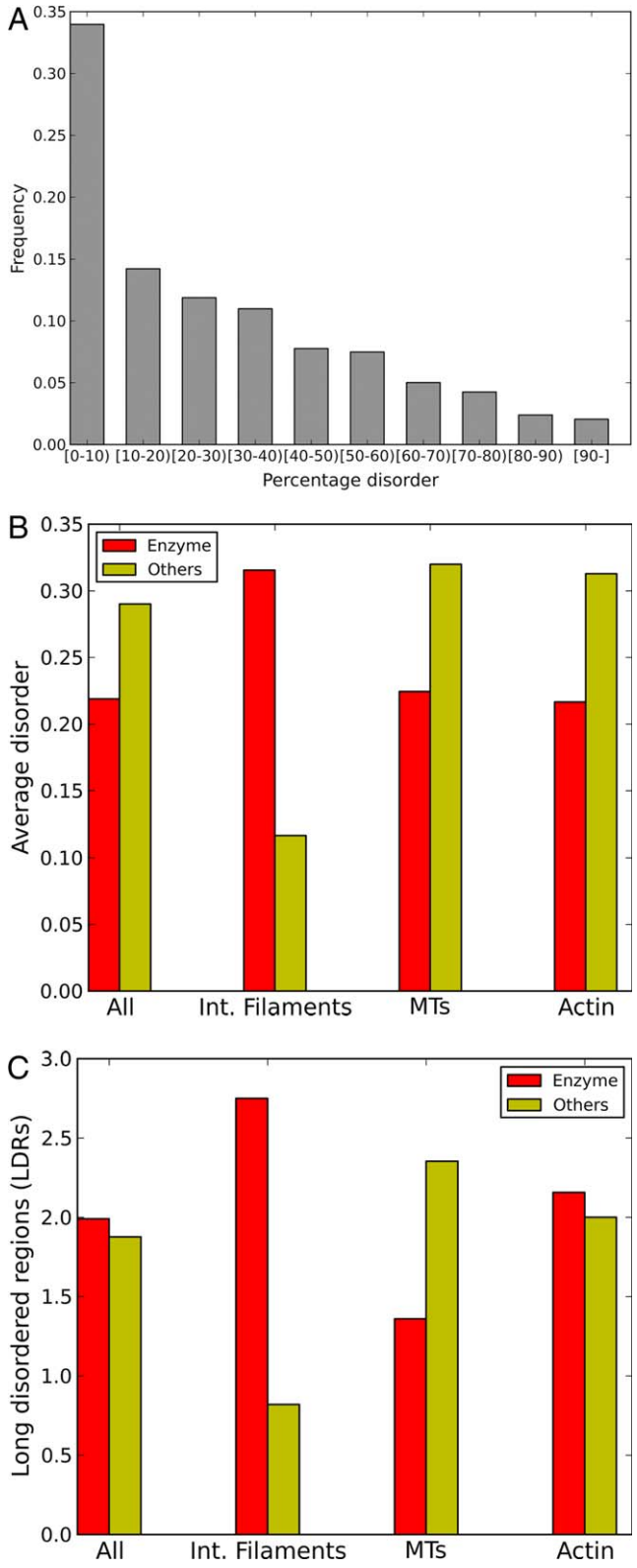

Fig. 1.

As outlined in great detail in the following sections, structural disorder often plays important roles in all three systems in: post-translational modification (tubulin tails, NF side-arms, practically all other accessory proteins), sequestration/stabilization of folded building blocks (actin: Tbeta4 [Safer et al., 1997], tubulin: stathmin [Wallon et al., 2000]), promotion of polymerization (MTs: microtubule-associated proteins (MAPS) [Dehmelt and Halpain, 2005], microfilaments: Spire and Cordon-Bleu [Renault et al., 2008]), providing a flexible spacer between filamentous core (NFs: side-arms [Brown and Hoh, 1997], MTs: MAPs [Mukhopadhyay and Hoh, 2001]), connecting to other elements (actin crosslinkers), targeting activity or signaling cascades (MTs: MAPs as A-kinase anchoring proteins (AKAPs) [Buday and Tompa, 2010]), creating a special physical microenvironment (NFs: phase transition of side-arms [Beck et al., 2012]) and much more complex regulatory relations. Structurally disordered accessory proteins are also involved in mediating the crosstalk between the different components of the cytoskeleton (average disorder 0.32, for 87 proteins involved with two or more cytoskeletal components, cf. also Table I).

Intermediate (Neuro) Filaments and Disorder

IFs constitute a principal filament system in metazoan cells [Fuchs and Weber, 1994] and IF proteins represent one of the most abundant cellular proteins. Within the cytoplasm and nucleus, they assume various flexible intracellular

Fig. 1. Distribution of disorder in the three cytoskeletal components. Disorder properties of all 1457 proteins involved in the cytoskeleton. (**A**) Histogram of predicted disorder (IUPred) shows a long tail in the distribution, (**B**) Average disorder of all proteins and separately for proteins involved with each filament type (IFs, MTs, and Actin). In addition, the proteins in each class are divided into two subcategories: enzymes and others. Enzymes are selected based on the presence of an EC number in the UniProt annotation. (**C**) Average number of long disordered regions (LDRs) for each protein subclass. LDRs are defined as 30 (or more) consecutive predicted disordered residues. Intervening stretches of upto three residues are ignored. In (B) and (C), standard deviations for all the bars are large, and therefore not plotted, so as to retain focus on the observed overall trends. In both plots, the number of proteins associated with the subgroups are: 210 (all_enzymes), 1247 (all_others), 8 (if_enzymes), 152 (if_others), 50 (mt_enzymes), 308 (mt_others), 172 (actin_enzymes), and 857 (actin_others); if = intermediate filaments, mt = microtubules.

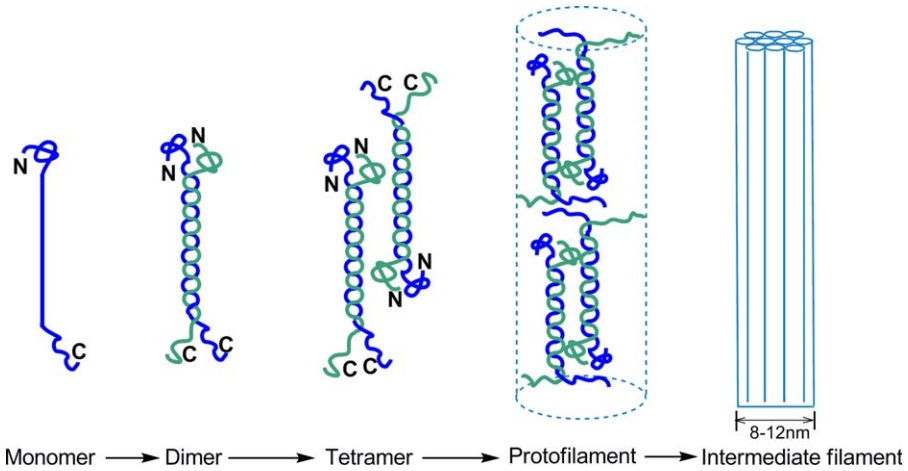

Fig. 2. Structural organization of IF fibers. Typical pathway of structural organization of intermediate filaments. A dimer of extensive coiled-coil structure forms of two monomers, forming a tetramer via lateral interactions and protofilaments via head-to-tail contacts. Disordered tail domain protrude from mature filaments and provide a platform for further interactions with accessory protein and post-translational modifications.

scaffolds depending on the cell type (Fig. 2). The IF network protects the cell against mechanical stresses [Lazarides, 1982] and plays role in several basic cellular processes (cell growth, proliferation and apoptosis) by interacting with various cellular proteins [Kim and Coulombe, 2007]. They were designated "intermediate" because their average diameter of 10 nm falls between thinner microfilaments (5–8 nm) and thicker MTs (25 nm). So far, about 70 genes

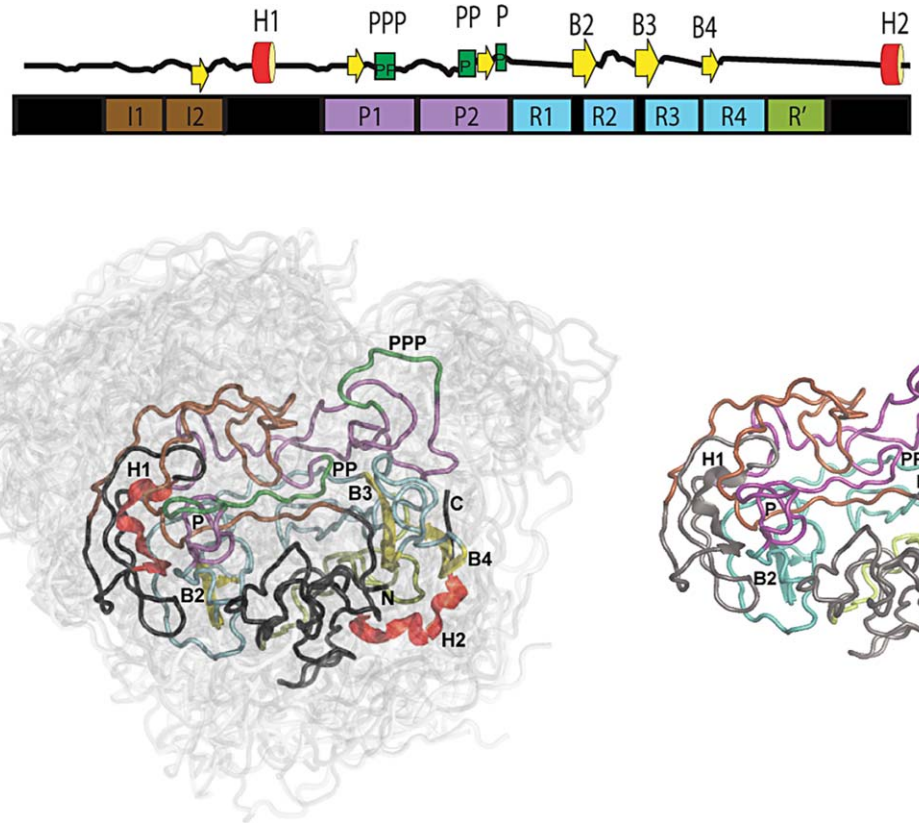

Fig. 3. NMR measurement and disorder prediction of tau protein. Major conformational features of human tau protein calculated from NMR data. The diagram above the domain structure shows the major transient short-range structural motifs observable: transient α-helical structure H1 and H2 (red cylinders), and β-structures, of which B2, B3, and B4 are highlighted (yellow arrows). Polyproline II stretches are shown as green boxes. In the lower panel, an ensemble of 20 conformations, with one highlighted by the secondary structural elements, is shown. The same conformation is also shown to the right, color coded according to the domain organization of tau. Adapted from PLoS Biology [Mukrasch et al., 2009] with permission.

belonging to the IF superfamily in six subfamilies were identified [Herrmann et al., 2003; Szeverenyi et al., 2008].

The genomic structure and the nucleotide sequence homology throughout the rod domain define the six major types (I–VI). The 28 type I and 26 type II intermediate filament proteins are the "acidic" and "basic" keratins, and account for most of the intermediate filaments (Table I). Keratins only assemble as heteropolymers: a type I and a type II protein form a heterodimer. There are four type III genes: desmin (muscle cells); vimentin (fibroblasts, lymphocytes, endothelial cells); peripherin, (peripheral neurons) and syncoilin. The seven type IV IF proteins are expressed mostly in nerve cells where they are implicated in the radial growth of the axon. The type V nuclear lamin IF proteins form intranuclear filaments. The type VI group includes the two eye lens intermediate filament, or "beaded filament", proteins CP49 (phakinin), and filensin (CP115).

The encoded proteins can be found in practically all cell types of the human body, but in neurons they are especially abundant (NFs). The conserved regions harbour a number of phenotypically pronounced point mutations in IF genes, which have been associated with at least 90 different diseases causing hair and nail defects, epithelial blistering disorders, heart or skeletal muscle abnormality, cardiomyopathies, neuropathies, and metabolic syndromes [Fuchs and Cleveland, 1998; Omary et al., 2004; Szeverenyi et al., 2008].

General Outline of IFs

IF components are much more diverse in their sequences than other cytoskeletal network elements, for example, MTs. Two well defined conserved regions can be identified across different IF proteins, both is located in an α-helical segment of the central rod domain and one is an absolutely conserved 13 amino acids long IF "consensus" motif, involved in dimer–dimer interactions within the mature filament [Herrmann et al., 2000]. Despite their diversity, members of the IF superfamily share similar patterns of secondary structure, dominated by a central rod domain and flanked by head and tail domains [Hertzog et al., 2004]. During IF formation, two parallel α-helical chains scroll into an extended coiled-coil dimer (Fig. 2). Following the head-to-tail association of the rods (usually 310 amino acids long) antiparallel protofibrils are formed. Two dimers join side-by-side to form a bidirectional, staggered antiparallel tetramer [Steinert et al., 1993; Strelkov et al., 2002], and mature IFs are assembled from these apolar tetramers forming so called "unit-length filaments" [Herrmann et al., 1996] by internal rearrangement of subunits and radial compaction of the filament [Herrmann and Aebi, 1999]. Although the ideal structural model of intermediate filament (Fig. 3) supposes eight tetramers in four distinct subfibrils, there is significant structural polymorphism among intermediate filaments [Goldie et al., 2007; Sokolova et al., 2006]. IFs are dynamic structures; several cross-linking proteins were identified to mediate interactions between intermediate filaments and the other cytoskeletal networks like plectin in vimentin fibers [Favre et al., 2011; Karashima et al., 2012] or filaggrin in keratin [Mack et al., 1993] (cf. Table I).

IFs are obligate heteropolymers composed of three subunits, IF-L(ight), IF-M(edium), and IF-H(eavy), which differ in their molecular weight (Mw) (68–70, 145–160, and 200–220 kDa, respectively). The central rod region is divided into four α-helical segments (1A, 1B, 2A, 2B) separated by three linker regions (L1, L12, L2) and flanked by nonhelical N-terminal head and C-terminal tail (CTT) domains [Fuchs and Weber, 1994]. The variable head and tail ends of IF proteins play key roles in the assembly, organization and regulation of intermediate filaments, for example, via post-translational modifications and interactions with other proteins [Kim et al., 2006]. The terminal regions that are predicted (Table I) and experimentally shown [Brown and Hoh, 1997] to be disordered, show wide variety in their length and sequence and are usually made up of three distinguishable regions. E1 (head) and E2 (tail) subdomains are highly charged; V1 (head) and V2 (tail) are variable domains containing loose repeat sequence motifs, and H1 (head) and H2 (tail) are "hypervariable" stretches that often contain phosphorylation target sites [Szeverenyi et al., 2008]. For example, the tail domain of IF-H contains more than 100 copies of a hexapeptide element, which harbours a characteristic KSP phosphorylation motif that contributes multiple sites for phosphorylation determining interfilament spacing [Brown and Hoh, 1997]. Phosphorylation of the head region can affect filament stability and it can also be involved in extensive cross-linking activities giving rise to hydrogel transitions [Beck al., 2012].

Neurofilaments are Special

Among IFs, NFs have unique properties. Three markedly different proteins called triplet proteins (NF-L, NF-M, and NF-H) constitute two morphologically distinct domains: core filaments and cross-bridges [Lee and Cleveland, 1996], the latter being only 3–5 nm in diameter. The NF triplet proteins are present in both the central and peripheral nervous system and are usually neuron specific. The carboxy-terminal domains of NF-M (∼60 kDa) and NF-H (∼200 kDa) extend from the filament backbone and project away from the filament as side-arms [Leapman et al., 1997], forming cross-bridges through noncovalent interfilament interactions [Chen et al., 2000; Nakagawa et al., 1995]. NFs fill the core of the axon with a characteristic interfilament spacing of 35–40 nm, which depends on the phosphorylation state and entropic exclusion of the tail domain [Brown and Hoh, 1997; Kumar and Hoh, 2004; Martin et al., 1999; Strong et al., 2001].

The tail domain of NF-H is longer and contains a multi-phosphorylation repeat domain with much more Lys-Ser-Pro (KSP) motifs, than NF-M [Pant et al., 2000], the serines of which are targets for phosphorylation. The level of phosphorylation varies within the cell; in distal regions of axons are the side-arms the most heavily phosphorylated and they are largely nonphosphorylated in perikarya and more distal regions of axons [Nixon et al., 1994]. The tails attain additional negative charges through serine phosphorylation which mediates the interaction between neighboring filaments, affects the organization of NF brushes and is considered to increase the lateral extension of sidearms [Martin et al., 1999]. Phosphorylation of NF-H side arms also regulates transport of NFs through axons [Ackerley et al., 2003; Lee et al., 2012].

Structural Studies

Due to their polymerization-prone character, IFs or IF proteins have not yet been crystallized. Rather, discrete domains or fragments are selected for crystallization and such structural data are now available for various fragments of vimentin, lamin A, and keratin, and also for the nonhelical tail domain of lamin A/C and vimentin.

The first pieces of structural information were obtained for vimentin, one of the best conserved IF proteins. Recently a human vimentin mutation has been linked to cataracts [Muller et al., 2009]. The molecular organization of human vimentin based on the crystal structures of three fragments [Strelkov et al., 2002] suggests that the fragment corresponding to segment 1A forms a single amphipatic α-helix, which might yield a coiled coil within an isolated dimer and is likely to play a role in specific dimer–dimer interactions during IF assembly. The 2B segment reveals a double-stranded coiled coil, which interferes heavily with IF assembly. The model could be later extended to the first half of its rod domain [Chernyatina et al., 2012] leading to an antiparallel tetramer model (cf. Fig. 2).

Nuclear lamins (also known as class V IFs) are special nuclear IFs, which form a two-dimensional matrix providing integrity and structural support for chromosomes and replicating DNA. Together with chromatin proteins and inner nuclear membrane proteins, they form the nuclear lamina which is essential for maintaining proper nuclear shape, spacing nuclear pore complexes and organizing heterochromatin [Stuurman et al., 1998]. The intertwining of lamin filaments and their carboxyl-terminal segments distinguish them from other IFs. Multiple alignments of the available amino acid sequences of lamins revealed two regions of high homology connected by a variable-length disordered linker [Krimm et al., 2002]. The first homology domain corresponds to the coiled coil rod domain common to all IF proteins, whereas the second C-terminal domain is unique to lamins and appears to be globular by electron microscopy [Stuurman et al., 1998].

Assembly of IFs

The role of head and tail domains in IF assembly has been studied by mutagenesis and in vitro assembly studies [Hatzfeld and Burba, 1994; Herrmann et al., 1996; Kouklis et al., 1993]. It is generally agreed that the head domain is more important in IF assembly than the tail domain; its deletion interrupts filament assembly at the dimer/tetramer stage [Beuttenmuller et al., 1994; Herrmann et al., 1996]. The tail domain containing the conserved TRDG motif is at least partially responsible for proper filament thickness [Makarova et al., 1994]. A study of the interaction between the isolated vimentin tail domain and actin containing structures suggested that the vimentin tail existed mainly in an extended conformation [Cary et al., 1994].

Although the role of structural disorder in IF function is recognized for some time [Ackerley al., 2003; Brown and Hoh, 1997], relatively little attention has been paid to the structural description of the flexible tail or head domain of IFs. Using site directed spin labeling and electron paramagnetic resonance (EPR), the structure and dynamics of the head domain of human vimentin [Aziz et al., 2010] and its tail domain in tetramers and filaments was studied and compared recently [Hess et al., 2013]. As opposed to head and rod domains, the tail domains are not closely apposed in protofilaments. More than half of the tail domain is very flexible in both the assembly intermediate and the intact IF: its first third, being a continuation of the central rod domain, is rather rigid and ordered, to transit abruptly to a more flexible, less ordered region (cf. Fig. 2) as shown by EPR. The tail domain is involved in protein/protein interactions that occur during filament elongation.

Involvement in Disease

97 distinct diseases have been associated with the IF gene family: inherited mutations affecting the primary structure of IF proteins are responsible for a vast number of inherited diseases and result in the formation of characteristic cytoplasmic inclusions [Fuchs and Cleveland, 1998; Wilson et al., 2001]. The majority of these genetic lesions are missense mutations affecting highly conserved residues at either the N- or the C-terminus of the central rod domain. Several hair, nail and skin defects were linked to mutations in the gene of type I or II keratins including epidermolysis bullosa and keratoderma disorders [Irvine and McLean, 1999]. Desminopathy is one of the most common intermediate filament human disorders associated with mutations in closely interacting proteins, desmin and alpha B-crystallin [Clemen et al., 2009]. In contrast to previous findings, where the disorder causing mutations were located mainly to the central region of IF proteins, in desmin tail domain mutations were as well described [Maddison et al., 2012]. Desmin is involved in several types of cardiomyopathy, too. The Charcot-Marie-Tooth disease and Parkinson's disease are

progressive neurological degenerations associated with mutations in NF genes. Laminopathies, the most phenotypically diverse group of IF gene related pathologies, are the collective term for diseases caused by mutations in the lamin genes [Wilson et al., 2001]. The role of vimentin and type VI IF proteins in autosomal dominant cataract has been published recently [Muller et al., 2009]; mutations in the gene encoding the carboxyl-terminal tail of Lamin A/C are associated with forms of muscular dystrophy and familial partial lipodystrophy [Wilson et al., 2001].

Microtubules

MTs are the largest of the filamentous cytoskeletal structures that pervade the cellular cytoplasm and help in the maintenance of cell shape, motility, divisions and intracellular transport. They are rigid, tubular filaments with a diameter of about 25 nm, built as a polymer of heterodimeric α/β tubulin subunits [Amos, 2000]. In cross-section, each MT is shown to consist of 13 individual (proto-) filaments assembled around a hollow core. Each protofilament is composed of a series of tubulin molecules that are linearly arranged with the same polarity (i.e., with identical head-to-tail orientation of the α/β-subunits), resulting in a plus (fast-growing) and a minus (slow-growing) end [Nogales, 2001]. MTs usually grow from specific nucleating sites in the cell (MT organizing centers), most commonly the centrosome, and nucleation involves a γ-tubulin variant [Kollman et al., 2011]. The minus ends of MTs are stabilized because they are embedded in the centrosome, whereas their plus ends grow outwards towards the cell boundaries. MTs undergo rapid cycles of polymerization and depolymerization (dynamic instability): this behaviour is regulated by GTP binding and hydrolysis [Howard and Hyman, 2009; Wade, 2009]. This inherent (dynamic) instability of MTs is carefully regulated by the cell (regulatory mechanisms include posttranslational modifications of the tubulin dimer, and the binding of MAPs) for specific functional purposes [Etienne-Manneville, 2010; van der Vaart et al., 2009].

Structural Aspects of the "Core" Microtubular Proteins

The MT core is composed of heterodimers of α- and β-tubulin, which have an N-terminal domain containing the nucleotide-binding region, an intermediate domain containing the taxol-binding site, and a C-terminal domain (PDBid: 1TUB) that ends in a highly acidic, disordered tail [Nogales et al., 1998], also observable in β-tubulin. NMR experiments [Lefevre et al., 2011], computational modeling [Freedman et al., 2011] and missing electron density for the CTT in crystal structures provide clear evidence for its highly flexible/disordered nature. The tubulin CTT protrudes from the MT surface and functions as the site of most of the post-translational modifications of tubulin [Sahab et al., 2012]. The CTT is also functionally important as it forms the binding site for a variety of tubulin/MT partners, including molecular motors [Wang and Sheetz, 2000], diverse MAPs (such as MAP2, tau and MAP4), and cations (such as Ca^{2+}) which are all major regulators of MT (dis)assembly and dynamics [Garnham and Roll-Mecak, 2012; Janke and Bulinski, 2011]. The variations of CTT among tubulin isotypes potentially explain the modulation of the dynamics of MT assembly in specific tissues or cytoplasmic regions.

The exterior of the MT shaft consists of the highly disordered, negatively charged tubulin tail. Post-translational modifications of the tubulin CTT create specialized MT surfaces that are geared towards manifold functions. Recent research has highlighted the large variety of tubulin modifications including Lys acetylation, arginylation, glutamylation, glycosylation, methylation, etc. [Wloga and Gaertig, 2010]. The observation that modified tubulin subunits are unevenly distributed along MTs has led to the hypothesis that the diverse post-translational modifications (PTMs) form a biochemical "tubulin code" that can be interpreted as a signal by MT interacting proteins/factors [Verhey and Gaertig, 2007]. The MT array can thereby be considered to comprise a block co-polymeric architecture composed of tubulin heterodimers, and with the PTMs of the tubulin building blocks marking MT subpopulations, thus selectively affecting downstream MT-based functions [Janke and Bulinski, 2011]. To add to the complexity, in PTMs such as poly-glutamylation and poly-glycylation, the specific length of the added chain can vary (glutamic acid chains are usually between 1 and 6; however, up to 20 have been observed). At neutral pH, the CTT negative charges cause it to remain extended due to the electrostatic repulsion within the tail, and between adjacent tails. These PTMs would not only differentially increase the chain length, but also alter the charge distribution and balance, all serving to fulfill (not all of which are understood at present) definite regulatory roles [Garnham and Roll-Mecak, 2012]. From the evolutionary standpoint, it seems logical that such regulatory functionalities were primarily added to the peripheral (exposed) tubulin tail, and not the tubulin body that is involved in "core" lattice interactions and where modifications would be likely to result in loss of viability. In accordance with what is known about the advantages of protein disorder, it is not surprising that this regulatory CTT segment is also highly unstructured.

Interestingly, the bacterial tubulin homolog FtsZ also has a marked disordered CTT (40–50 residues). FtsZ is present ubiquitously in eubacteria, archaebacteria and has also been identified in chloroplasts [Erickson, 1997]. As with tubulin, FtsZ also serves a cytoskeletal role as demonstrated by its formation of protofilament sheets and mini-rings that serve as the cytoskeletal framework for a contractile ring structure (Z ring) at the future cell division site [Erickson et al.,

2010]. The timing and the location of cell division is regulated by ring assembly. The FtsZ ring further recruits other cell division proteins to the septum to produce a new cell wall between the daughter cells. Homodimerization of FtsZ requires the central region and the disordered CTT, which is the first step towards polymerization and formation of the dynamic Z ring. Critical to cell division, the Z ring formation is under tight regulation, and FtsZ has multiple binding partners. It is increasingly evident that a conserved stretch of amino acids at the CTT of FtsZ is involved in many of these interactions; evidence has been reported for the interaction of the CTT with MinC, FtsA, EzrA, ClpX, and SepF. Mutations in the C-terminal conserved core have been described that abolish binding to one or more partners [Krol et al., 2012]. Although disordered in the unbound form, recent crystal and NMR structures demonstrate the functional role of disorder in the binding via disorder to order transitions. Bound to ZipA, the C-terminal peptide forms an extended β-strand followed by an α-helix, whereas bound to FtsA the peptide is predominantly helical (thereby demonstrating that the FtsZ CTT can adopt different conformations to fit different binding partners).

Microtubule-Associated Proteins (MAPs)

Research over several decades has resulted in an expanding list of MAPs, knowledge of their phosphorylation states, and their effects on MT dynamics and regulation [Mandelkow and Mandelkow, 1995]. Several types of MAPs have evolved in eukaryotes, including structural MAPs, microtubule plus-end-binding proteins (+TIPs) and MT motors (cf. Table I).

"Structural" MAPs

"Structural" (or assembly-) MAPs bind to, stabilize and promote MT assembly. These proteins share a conserved C-terminal domain containing MT-binding repeats, and a variable projection domain (that serves to scaffold MTs with other cellular proteins, intermediate filaments, membrane components and neighboring MTs). In electron micrographs, the projection domain appears as a filamentous arm extending from the MT wall.

Based on sequence features, MAPs have two main families: Type I (MAP1A/1B) and Type II (MAP2, Tau, and MAP4), and several isoforms generated by alternative splicing. MAP1A/1B are large, filamentous proteins found in axons and dendrites of neurons and also in non-neuronal cells [Halpain and Dehmelt, 2006]. Structural details of MAP1-family proteins are largely unknown. Electron microscopy studies have however suggested their elongated, flexible shape [Sato-Yoshitake et al., 1989; Shiomura and Hirokawa, 1987] and disorder predictions indicate that both MAP1A and 1B are extensively disordered (Table I). They contain basic KKEX (Lys-Lys-Glu-X) repeats that bind to negatively charged tubulin [Noble et al., 1989] and potentially reduces charge repulsion between tubulin subunits within MTs, thus stabilizing the polymer.

Type II MAPs include MAP2 and Tau (found in neurons), and the ubiquitous MAP4 (present in neuronal and many non-neuronal tissues) [Dehmelt and Halpain, 2005]. In mature neurons Tau is present mainly in axons whereas MAP2 is restricted to cell bodies and dendrites. MAP2 and Tau can form fibrous connections (cross-bridges) between MTs and form stable MT bundles. MAP4 is thought to regulate MT stability during mitosis. Type II MAPs possess three or four repeats of an 18-residue stretch in the MT-binding domain [Al-Bassam et al., 2002]. Reversible phosphorylation of MAPs promotes MT disassembly because phosphorylated MAPs are unable to bind to MTs, a structure occurs with Tau in Alzheimer's disease, for example [Gong and Iqbal, 2008].

MAP2 and Tau (Fig. 3) proteins are intrinsically disordered [Mukrasch et al., 2009]. All MAP2/Tau family proteins have MT-binding repeats near the C-terminus [Goedert et al., 1991], each containing a conserved KXGS motif that can be reversibly phosphorylated. These repeats constitute the "core" MT-binding region that undergoes disorder-to-order transition during MT binding. The N-terminal projection domain has a net negative charge and exerts a long-range repulsive force [Mukhopadhyay and Hoh, 2001], thus behaving as "entropic chains" to regulate inter-MT spacing in axons and dendrites [Chen et al., 1992]. Functional orthologs of MAP2/Tau proteins are found in diverse organisms such as *C. elegans* (PTL-1) and *D. melanogaster* that are also predicted 100% disordered. The nature and behavior of protein disorder in case of Tau has been extensively characterized by several biophysical techniques (Supporting Information Table S2) [Narayanan et al., 2010]. NMR secondary chemical shifts and dipolar couplings detect β-structure propensity within the MT-binding four-repeat region and largely random coil structure in the flanking domains. Chemical shift perturbation experiments also identify motifs in both the upstream and downstream flanking domains, (225)KVAVVRT(231) and (243)LQTA(246) respectively, that strongly contribute to the binding to the acidic MT exterior. This model is consistent with the "jaws" model of Tau-MT interactions and clearly highlights the importance of the disordered regions for both MT binding and pathological Tau aggregation [Mukrasch et al., 2007].

In addition to MT binding and stabilization, these MAPs also modulate cargo transport and regulate MT dynamics by performing adaptor functions by anchoring signaling proteins in an adaptive binding process [Gundersen and Cook, 1999]. Binding of MAP2 to the RII regulatory subunit of PKA is a very well-characterized example of a classical MAP functioning as an adaptor protein [Obar et al., 1989]

due to which they belong to the family of A-kinase anchoring proteins (AKAPs).

MT Plus-End Tracking Proteins (+TIPs)

+TIPs constitute a structurally and functionally diverse protein family whose members specifically bind to and accumulate at the plus ends of MTs [Akhmanova and Steinmetz, 2010], which is highly dynamic and undergoes alternating phases of growth and shrinkage (catastrophe). A variety of intracellular processes critically depend on MT dynamics in which +TIPs play important roles. +TIPs exhibit a limited set of evolutionarily conserved linear motifs, which feature in domain-linear motif-mediated interactions that interface the MT system with other cellular structures and signaling networks [Akhmanova and Steinmetz, 2008]. These recognition regions typically appear within regions of predicted disorder which are involved in specific but reversible (with low micromolar affinities) binding typical of IDPs. +TIPs have four major classes (cf. Table I).

(1) End-binding (EB) family: the members contain a strongly conserved N-terminal calponin homology (CH) domain [Hayashi and Ikura, 2003] and an adjacent linker region which cooperate in binding MT plus ends. The C-terminal region consists of an α-helical coiled-coil domain, an EB-homology domain and an acidic tail bearing an EEY/F linear motif. The EBH domain and the acidic EEY/F motif act as interaction hubs and enable EB proteins to interact with a gamut of other +TIPs and recruit them to MT ends, typical of the moonlighting capacity of IDPs/IDRs [Tompa et al., 2005].
(2) Cytoskeleton-associated protein glycine-rich (CAP-Gly) domain: this is a small globular domain containing an evolutionary conserved hydrophobic cavity and multiple Gly residues at specific locations. CAP-Gly domains use their apolar cavity to interact with MTs and the consensus EEY/F sequence motifs of EB family proteins. The best-studied members of this family are CLIP-170 and the dynactin complex p150glued. Disorder predictions for p150glued show that the CAP-Gly domains (regions 78–120, 232–274) are ordered, but there are other long disordered regions in the protein (cf. Table I).
(3) SxIP motif-containing +TIPs: These are characterized by low-complexity sequence regions enriched in basic, serine and proline residues that form the SxIP-motif. This motif acts as a general "microtubule tip localization signal" (MtLS) that is specifically recognized by the EBH domain of EB-family proteins and thereby causes these proteins to be recruited to the MT-plus ends. Well-characterized members include the adenomatous polyposis coli (APC) tumor suppressor, the spectraplakin microtubule-actin crosslinking factor (MACF1) and the mitotic centromere-associated kinetin (MCAK). Disorder predictions of all these proteins show several long disordered regions (cf. Table I).
(4) TOG/TOG-like domain containing proteins: this class includes members of the XMAP215 and CLASP families that play central roles in the regulation of interphase MT dynamics and the proper formation of mitotic spindle architecture and flux. Their characteristic feature is the presence of tandem arrangement of TOG domains that enable binding to tubulin [Slep, 2009]. CLASPs additionally contain SxIP-motifs that enable plus-end binding and stabilization.

MT Destabilizers

The Op18/stathmin family are well characterized IDPs that destabilize MTs and increase their turnover, thus making possible rapid reorganization of the microtubular cytoskeleton [Cassimeris, 2002]. This destabilization occurs either by stathmin causing the sequestration of tubulin dimers, or by the stimulation of MT plus-end catastrophes. In solution, free Op18/stathmin has negligible secondary structure, existing in a rapid equilibrium between a disordered ensemble and a state more structured containing a long α-helical structure [Steinmetz et al., 2000]. Binding to tubulin stimulates folding of a large region of Op18/stathmin into a long, extended α-helix, with the terminal region inhibiting elongation of the polymer (Supporting Information Table S2).

Microtubular Motor Proteins

Another broad class of MAPs are motor proteins that use MT as "railway tracks" in intracellular transport [Mallik and Gross, 2004]. Two large superfamilies have been identified: kinesins transport cargo towards plus ends of MTs, whereas dyneins drive minus end-directed retrograde transport. Both motors consist of two heavy chains and several light chains. Each heavy chain is composed of a conserved, globular ATPase head domain, and an elongated tail region. Kinesin is the founding member of the diverse kinesin superfamily [Hirokawa et al., 2009]. It contains two identical approximately 960-residue heavy chains containing an N-terminal globular motor domain, a central α-helical stalk that enables dimer formation through a coiled-coil, and a CTT (Fig. 4A) that is both autoregulatory (binds to the motor domain to inhibit its ATPase activity), and also responsible for specific cargo selection [Karcher et al., 2002]. The adaptor domains are structurally diverse thus enabling specific cargo selection. Intrinsic disorder is a common structural feature of nonmotor domains of kinesins (cf. Table I) [Seeger et al., 2012], confirmed by CD and

NMR structural studies [Seeger et al., 2012]. The disordered nature of these tail regions plays an important role in facilitating cargo recognition and conferring functional specificity to kinesins (cf. Table I).

Several insights can be obtained by comparing available structural data for molecular motors with disorder predictions. In kinesin, for example, ~20% of the motor domain is predicted as disordered (Fig. 4B), within loops 1, 2, 7, 10, and 12, the P-loop, Switch I and II, and the neck-linker. Several of these structures undergo conformational changes in response to events such as nucleotide exchange and/or MT-binding and release [Kull and Endow, 2002; Sindelar and Downing, 2010]. Cryo-electron microscopy (EM), electron paramagnetic resonance spectroscopy (EPR), Forster resonance energy transfer (FRET), etc. studies indicate a large-scale conformational change in the disordered neck-linker region following ATP and MT-binding, thus demonstrating the role of structural disorder in the communication link between the ATP and MT-binding sites and the neck linker [Vale et al., 2000].

The mechanism of powered motion requires an important disorder-to-order transition as elegantly demonstrated by two kinesin structures (PDBid 1bg2: before; and, 2kin: after the power stroke), which show how a small change due to ATP-ADP transition results in a large structural change in the motor. A small change in the ATP-binding region upon ATP hydrolysis pushes on the relay helix causing it to form a perfectly sized pocket for the neck linker. Before the power stroke, the pocket is too small and the linker is disordered; after the power stroke, the pocket attains the correct size for the neck linker to zipper into the protein (Fig. 4C), dragging along the neck and any attached cargo.

The structures of kinesin stalks are more complex than an uninterrupted coiled-coil. In case of the kinesin motor Kif5B, for example, the stalk contains several short flexible hinge regions that are predicted to be disordered and enable the molecule to fold into a compact conformation under

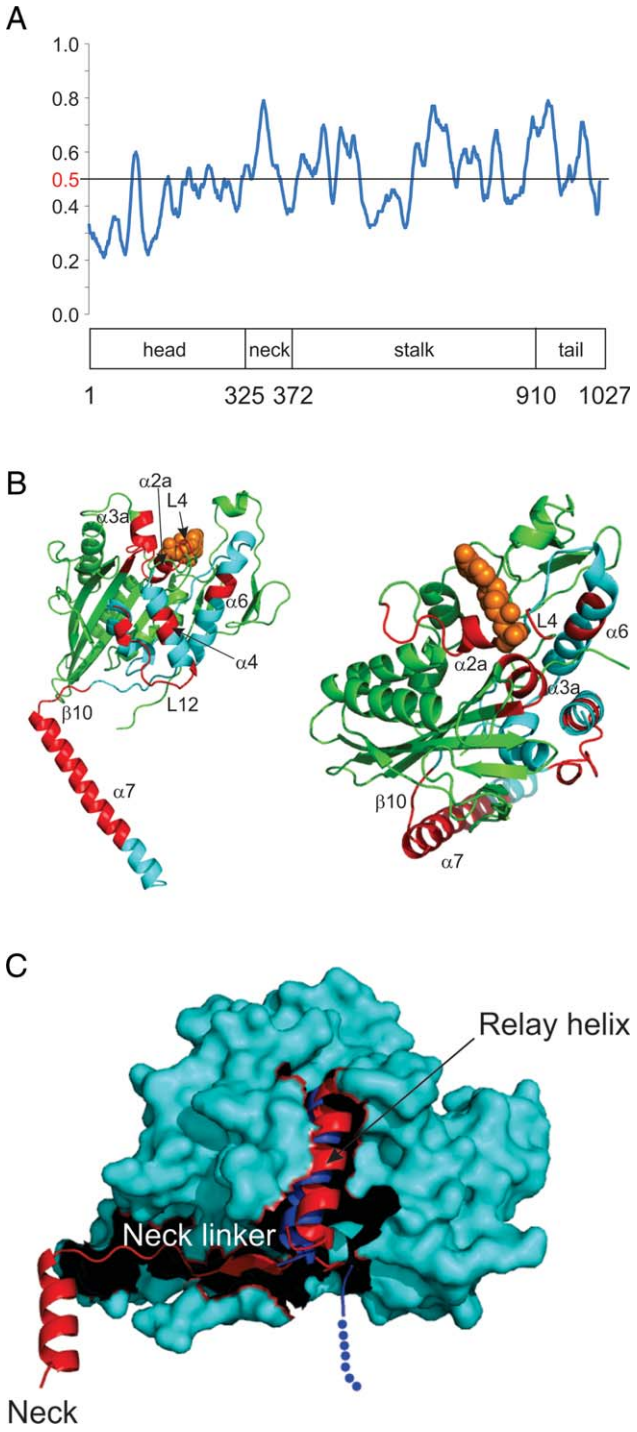

Fig. 4.

Fig. 4. Examples of structural disorder in kinesin motor structures. (**A**) Domain organization and predicted disorder of kinesin (UniProtID: Q6QLM7): values on the *y*-axis represent predicted disorder (RONN [Yang et al., 2005]). The value of 0.5 represents the cutoff and residues with values higher than 0.5 are predicted disordered, (**B**) Cartoon representation of the kinesin structure (PDBid: 3KIN) consisting of the head domain and neck linker region. Segments colored red are predicted disordered (according to values plotted in Fig. 4A), and they are labeled according to the structural nomenclature used by Kozielski et al. [Kozielski et al., 1997]. The ADP molecule is drawn in orange spacefill. (**C**) Disorder-to-order transition in the neck linker of kinesin during the power stroke. Surface representation shows the kinesin structure (PDBid: 2KIN) in cyan, with the 'relay' helix, the neck linker and the neck start region drawn as cartoon (red color). Superposed on it is another structure of kinesin (PDBid: 1BG2), for which only the relay helix and the CTT at the start of the neck linker sequence are shown in blue (the neck linker itself is disordered, shown with dotted lines, and is missing from the crystal structure). The rest of the structure has been omitted for clarity. Overall the two structures superpose with a Cα RMSD of 1.97 Å, and the largest deviations are seen in the relay helix structure. The ATP binding site is on the opposite face of the molecule and cannot be seen in this view. ATP hydrolysis causes a subtle conformational change in the structural elements forming the ATPase active site, that is transmitted via the relay helix and results in restructuring the neck linker binding cleft such that the neck linker undergoes a disorder (blue dots) to order (red cartoon) transition.

certain conditions [Stock et al., 1999]. Similarly, the stalk of Kif10 is over 2000 residues long, and predicted to be mainly coiled-coil with multiple, distinct 10–100 residue-long regions of predicted disorder. Kinesin-11 proteins are predicted to have a nearly 100% disordered stalk encompassing ~1000 residues [Seeger et al., 2012]. Several kinesins form coiled-coil heterodimers using their stalk regions, and the critical feature that evidently enables dimer formation is the interaction of patches of intrinsically disordered, oppositely charged residues in the associating monomer stalks [Chana et al., 2005].

The tail domains of several kinesins are also significantly disordered (varying in length from tens to hundreds of residues) and represent the most variable regions of the motors. These regions might be involved in motor domain autoregulation, posttranslational modifications [Guillaud et al., 2008], and interaction with specific cargoes [Hirokawa et al., 2009]. The C-terminal disordered tail of the Kif5B kinesin motor can bind more than 15 unique partners (including cargo proteins such as Syntabulin, RanBP2, SNAP 25/23, p180).

Cytoplasmic dynein is another important MT-based motor that is composed of multiple heavy, intermediate and light chains. The intermediate chains (IC) have critical roles in dynein assembly, regulation and cargo binding. The N-terminal region of ICs bind to diverse light chains and cellular cargo; this region is intrinsically disordered, and undergoes induced folding upon binding to the light chains LC8 and Tctex-1 [Benison et al., 2006] (Supporting Information Table S2). Multipartner binding interactions is also the primary function of dynein light chain protein LC8. This protein can bind over 22 different proteins, and these distinct interactions are all accommodated while maintaining binding specificity through specific interactions between disordered residues on both the LC8 and its ligands [Nyarko et al., 2011].

Involvement in Disease

The MT system—or its components—are involved in distinct diseases. Tau is implicated in Alzheimer disease where the neuronal cytoskeleton in the brain is progressively disrupted and replaced by tangles of paired helical filaments (PHF) mainly composed of hyperphosphorylated Tau [Zheng-Fischhofer et al., 1998]. Defective Tau also causes frontotemporal dementia, characterized by presenile dementia with behavioral changes, deterioration of cognitive capacities and loss of memory. The MT system is also involved in cancer, because disrupting MT dynamics affects mainly rapidly dividing cells, which is why small molecules such as Paclitaxel, Taxotere etc. are potent agents for chemotherapy [Jordan and Wilson, 2004]. Defects in cytoplasmic dynein can cause Charcot-Marie-Tooth disease [Weedon et al., 2011], characterized by progressive muscle weakness and atrophy. It can also cause mental retardation autosomal dominant type 13, characterized by below average intellectual functioning and behavioral impairments. APC protein is involved in familial adenomatous polyposis, contributing to tumor development and characterized by adenomatous polyps of the colon [Rustgi, 2007] and rectum, but also of upper gastrointestinal tract. APC disregulations also the cause of gastric cancer, mismatch repair cancer syndrome, and medulloblastoma. Defects in dynactin are the cause of progressive lower motor neuron disease: a neuromuscular disorder and Parkinsonism with alveolar hypoventilation and mental depression (Perry syndrome), and also susceptibility to amyotrophic lateral sclerosis [Rustgi, 2007].

Actin Cytoskeleton

In eukaryotic cells, actin is the most complex, flexible and versatile cytoskeletal component responsible for motility, endocytosis, intracellular trafficking, and cell morphology (Fig. 5). Actin filaments and monomers interact with an abundance of actin-binding proteins (ABPs) that organize actin networks in the cell, connect the actin structures to other parts of the cytoskeleton, and function in intercellular signaling [dos Remedios et al., 2003]. Actin is highly abundant in eukaryotic cells and extremely well conserved among species. Vertebrates usually have six different isoforms of actin: α-cardiac muscle actin, α-skeletal muscle actin, α-smooth muscle actin, β-cytoplasmic actin, γ-cytoplasmic actin, andγ-smooth muscle actin, which differ only slightly in their amino acid sequences [Vandekerckhove and Weber, 1978], and their expression pattern varies between tissue types and developmental stages [Tondeleir et al., 2009].

Actin exists in cells in two forms: the monomeric globular G-actin and the filamentous F-actin in the shape of a two-stranded helix [Oda et al., 2009]. Frequently they form flexible structures such as filopodia or lamellipodia (Fig. 5) that help exploring the environment or produce movement in the absence of motor proteins [Mattila and Lappalainen, 2008]. More rigid actin filaments are for instance stereocilia, found at the surface of hair cells in the inner ear and serve as detectors for sound [Tilney et al., 1983]. Inside cells, actin is generally located in the cytoplasm and, to some extent, also in the nucleus. The major contractile structures in many nonmuscle cells are stress fibers, bundles formed from cross-linked actin filaments together with myosin II, which function in mechanotransduction as focal-adhesion-anchors.

Actin polymerization is a tightly regulated [Gieni and Hendzel, 2009] dynamic process where ATP-actin is incorporated at the barbed end of the filament while ADP-actin dissociates from the pointed end [Pollard, 1984]. Each subunit is an enzyme that catalyzes the hydrolysis of ATP to ADP accompanied by a conformational change, allowing ABPs to distinguish between the ATP and the ADP forms

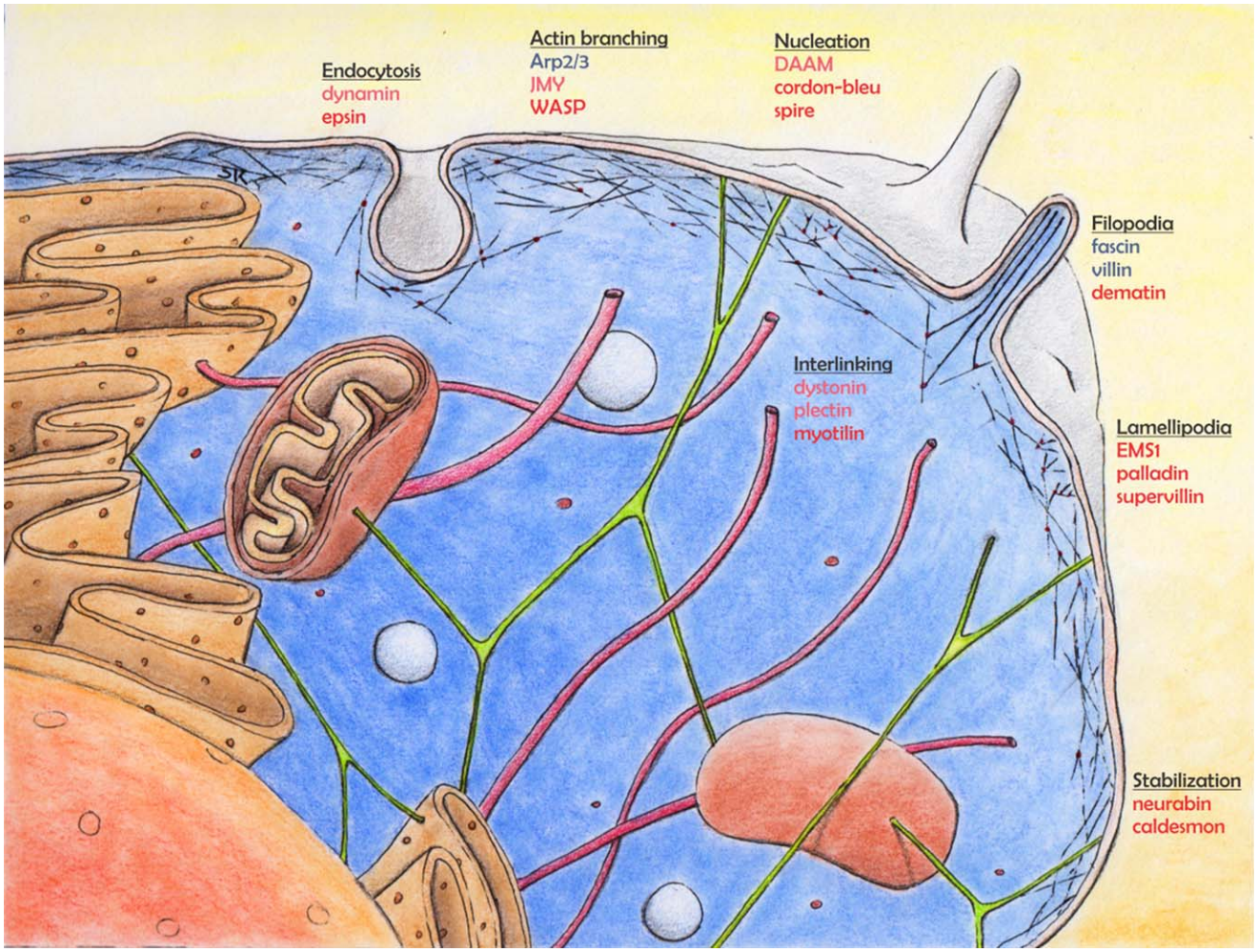

Fig. 5. Structural disorder in actin regulatory proteins. Animal cell with nucleus (orange, left bottom), endoplasmic reticulum (brown, above the nucleus), vesicles, mitochondria and components of the cytoskeleton: MTs (red), intermediate filaments (green), and actin filaments (thin black lines) close to the cytoplasmic membrane. Red dots on the actin filaments indicate Arp2/3 complexes involved in branching. ABPs are involved in regulating all aspects of the function of actin cytoskeleton: they are indicated close to the sites of processes they are involved in. The ABPs are color coded according to the degree of disorder: no disorder, less than 20% disordered residues (blue), between 20 and 50% disordered residues (pink), and more than 50% disorder (red).

[Graceffa and Dominguez, 2003]. Treadmilling, that is, polymerization driven by ATPase activity, allows for dynamic behavior of the structural system and thus for cell motility at the cost of ATP energy [Oda et al., 2009; Wegner, 1976]. Nucleation and growth of new filaments is the limiting step in F-actin formation as it is energetically unfavorable until three monomers or more associate [Winder and Ayscough, 2005]. New filaments can also branch out or severe from an existing filament, which enables subtle control of filament formation in cell movement, morphology and muscle contraction [Winder and Ayscough, 2005].

Nucleation can be initiated via three different mechanisms that are catalyzed by three main classes of proteins: the Arp2/3 complex together with nucleation promoting factors (NPFs), formin family proteins, and tandem W domain-based filament nucleators [Dominguez, 2010; Firat-Karalar and Welch, 2011]. All of them initiate filament growth by forming a stable actin trimer as nucleus and, more interestingly, all three mechanisms utilize proteins with IDRs (cf. Table I) [Goley and Welch, 2006; Sitar et al., 2011; Xu et al., 2004].

The Arp2/3 Complex Nucleates Actin Filaments with the Help of ABPs

The Arp2/3 complex generates y-branched actin networks by mimicking the critical trimeric nucleus and subsequently stabilizing filament growth by serving as a pointed-end–capping protein (CP) [Goley and Welch, 2006]. Y-branched actin networks are found in lamellipodia and thus involved in cell movement. During the nucleation, the binding of an actin monomer, mother filament and an activator NPF stabilize flexible Arp2 subdomains and hydrolysis of ATP can take

place [Nolen et al., 2004]. In vivo, several NPFs such as WASP can recruit Arp2/3 and actin for de novo nucleation. The WASP-Homology 2 (WH2 or W) domain, a small, disordered actin-binding motif is present in all NPFs and tandem W domain-based filament nucleators [Beck et al., 2012; Dominguez, 2010]. The molecular recognition effector role of disordered segments in WASP that function via a disorder to order transition has been demonstrated by crystallography [Kim et al., 2000] (Supporting Information Table S2). In NPFs one or more W domains are coupled with C (central or connecting) and A (acidic) motifs that bind subunits of the Arp2/3 complex and stabilize it in its activated conformation. A SAXS study of an activated complex consisting of the Arp2/3 complex, the verprolin homology domain or WASP2 homology 2 domain, cofilin homology domain, and acidic region (WCA) of N-WASP and one actin monomer shows a model of activation clearly depending on the inherent flexibility of the WCA motif to connect Arp2/3 with G-actin [Boczkowska et al., 2008]. Other WCA carrying members of this group (also called class I NPFs), are WASH, WHAMM, SCAR/WAVE, and JMY [Dominguez, 2010; Rottner et al., 2010] (cf. Table I). The class I factor JMY (junction-mediating and regulatory protein) is frequently described as one of the tandem W domain-based filament nucleators due to its ability to nucleate actin filaments in the presence and absence of Arp2/3 [Rottner et al., 2010] by "monomer-clustering" similar to Spire and Cordon-bleu, where several actin monomers are arrayed along a stretch of WH2 repeats [Sitar et al., 2011; Zuchero et al., 2009]. Actin itself can also be present in the nucleus where it might be involved in transcription regulation [Philimonenko et al., 2004].

Class II NPFs, however, contain the acidic Arp2/3 binding domain but possess an F-actin binding region. In the case of cortactin (Table I), binding to F-actin occurs through the central cortactin repeats, a molten globule domain that presumably undergoes ligand induced folding [Shvetsov et al., 2009]. The actin binding domain consists of four to six repeats made up of 37 amino acids connected to a SH3 domain by a disordered proline-rich region containing regulatory phosphorylation sites [Weed et al., 2000]. The SH3 domain facilitates binding to other ABPs containing a conserved prolin-rich motif such as N-WASP [Mizutani et al., 2002]. When activated, cortactin recruits Arp2/3 complex proteins to existing actin microfilaments.

Other members of the class II NPFs include Abp1 and Pan1 that bind F-actin through the structured actin-depolymerizing-factor homology or a coiled coil domain, respectively [Goley and Welch, 2006].

Tandem W Domain-Based Assisted Filament Nucleation

A second class of nucleating proteins that utilize the WH2 motif is the tandem W domain-based filament nucleators like Spire, Cordon-bleu (Cobl) and leiomodin [Dominguez, 2010; Goley and Welch, 2006; Sitar et al., 2011], which promote the growth of nonbundled, unbranched actin filaments. The common nucleation mechanism of these proteins is via the formation of a filament-like polymerization nucleus [Dominguez, 2010]. The N-terminal domain of Spire, for example, binds four actin monomers like beads on a string with its W domains until they form a polymerization nucleus of the shape of one strand of the long-pitch helix of the actin filament [Sitar et al., 2011]. The disordered linkers between the W domains are rather short in Spire (~10 amino acids) while the brain-enriched Cobl has a 65 amino acid long proline-rich linker between two of its three W domains giving Cobl a stronger nucleation activity [Ahuja et al., 2007]. Cobl forms and stabilizes, therefore, an actin trimer with the third monomer in cross-filament orientation.

Formins Nucleate Unbranched Filaments

Formins, the third type of nucleators, are generally implicated in the assembly of unbranched filaments, cytokinetic contractile rings, filopodia, and adherens junctions. They are large, multidomain proteins with significant sequence variability. The best studied members, mDia, Bni1, Bnr1, and DAAM have similar domain architecture and contain a intrinsically disordered GTPase-binding domain that adopts helical conformation upon interaction with the GTPase [Dominguez, 2010]. Formins surround the fast-growing barbed end of filaments and remain associated with them. Critical domain for filament nucleation is the FH2 domain which forms a unique "tethered dimer" with a flexible lasso and linker structure that allows the FH2 to "stair-step" on the barbed end while elongating the filament [Xu et al., 2004].

ABPs in Actin Filament Growth and Organization

Actin filament growth is regulated by a wide range of ABPs which frequently employ WH2 domains for G-actin binding [Paunola et al., 2002], but regulate growth and branching of the actin cytoskeleton differently. While WASP feeds actin monomers into the growing filament, polymerization antagonists such as thymosins bind G-actin to sequester it [Paunola et al., 2002]. Disordered thymosin β4 is mainly expressed in neurones and oligodentdrocytes and its main function is to bind and sequester actin monomers [Safer et al., 1997].

Growth of the actin cytoskeleton is generally regulated by F-actin binding proteins such as capping proteins that control filament length or cross-linking proteins that organize the filaments into bundles or networks. These ABPs frequently utilize IDRs to simultaneously interact with the

filaments and other associated proteins (Table I). The pointed-end-CP tropomodulin, for example, stabilizes F-actin in myofibrils in muscle sarcomers [Kostyukova et al., 2001; Uversky et al., 2011]. Structural studies identified a globular C-terminal domain and an intrinsically disordered N-terminus that contains three binding sites: two tropomyosin–binding sites and a tropomyosin-dependent actin-capping site [Kostyukova et al., 2001]. The flexible actin CP suppresses actin polymerization at the barbed end by binding it with its so-called β-tentacle, a C-terminal region that only forms a stable amphipathic helix when it binds to the hydrophobic cleft of actin [Takeda et al., 2011; Zwolak et al., 2010]. A number of proteins (CARMIL proteins) carry a CP-binding motif in a disordered region, and are able to inhibit CP by dramatically decreasing its affinity for the barbed end [Uruno et al., 2006].

Intrinsically disordered domains and motifs are also frequently employed by cross-linking ABPs. Intrinsically disordered caldesmon, one of the most abundant proteins detected in smooth muscle and in a number of nonmuscle cells, has a functionally important C-terminal domain [Permyakov et al., 2003]. Caldesmon cross-links thick and thin filaments by binding actin filaments and myosin [Morgan and Gangopadhyay, 2001]. While its N-terminal part has been described as a myosin/calmodulin-binding domain, the C-terminus contains a tropomyosin/actin/calmodulin-binding domain [Permyakov et al., 2003]. Myotilin, a component of a complex of multiple actin cross-linking proteins that belongs to the palladin family, has a unique N-terminal IDR [Salmikangas et al., 2003]. The protein is involved in the control of myofibril assembly and stability at the Z lines in muscle cells and has been implicated in muscular dystrophy [Salmikangas et al., 2003]. Palladin itself was found to localize at sites where active actin remodeling takes place, such as lamellipodia [Otey et al., 2005].

A number of proteins involved in actin filament organization and nucleation play a critical role in the formation of cellular protrusions such as filopodia and invadopodia. Examples include the afore-mentioned cortactin and supervillin which belongs to the villin/gelsolin family of actin-organizing proteins [Silacci et al., 2004] which also contains the members dematin and gelsolin that possess IDRs of 315 and 40 residues, respectively [Chen et al., 2009; Smirnov et al., 2007]. Supervillin has a unique, more than 800 amino acid long, intrinsically disordered N-terminus which promotes interactions with several signaling proteins and major cytoskeletal components, including F-actin and human nonmuscle myosin II [Chen et al., 2003; Crowley et al., 2009; Fedechkin et al., 2012] and it influences cytokinesis, cell motility and can promote invasive activity in tumors by formation of invadopodia or podosomes [Crowley et al., 2009; Weaver, 2006]. Invadopodia and podosomes are actin-rich protrusions that form at sites of extracellular matrix (ECM) degradation [Weaver, 2006]; tumor cells forming the highly active invadopodia are particularly invasive and migratory [Weaver, 2006].

Aside from regulating the polymerization and organization of actin filaments, actin and ABPs have a variety of other functions in endocytosis and trafficking [dos Remedios et al., 2003; Mooren et al., 2012]. Several of the proteins involved in endocytosis, such as dynamin, epsin, or auxilin, interact with actin and have been reported to have IDRs to facilitate effective vesicle formation [Dafforn and Smith, 2004; Gu et al., 2010]. In all, intrinsic disorder is involved in the function of most actin-regulatory proteins.

Intrinsically Disordered ABPs in Disease and Infection

The influence of ABPs on cell mobility, signaling and trafficking by small binding motifs such as the W domain is frequently implicated in cancer and pathogens [Condeelis et al., 2005]. Many bacterial pathogens exploit the host Arp2/3 complex to induce actin polymerization once they enter the host cytoplasm. *Listeria monocytogenes* expresses proteins that mimic WASP family proteins but lack their regulatory domains, for example, the IDP Listeria ActA binds actin and promotes constitutive actin nucleation [Footer et al., 2008]. Other proteins encoded by the pathogens have the ability to recruit and activate host ABPs to influence cytoskeleton structure and dynamics. The malaria parasite Plasmodium falciparum, for instance, remodels the actin cytoskeleton of host cells [Gomes-Santos et al., 2012; Radtke et al., 2006] by upregulating gelsolin, a growth regulatory ABP that prevents filament elongation [Gomes-Santos et al., 2012]. Pathogenic bacteria express proteins that directly interact with the host actin cytoskeleton. The Salmonella invasion protein A (SipA), a virulence factor, binds to actin and influences cytoskeletal rearrangement to promote uptake of the pathogen. SipA employs two IDRs described as nonglobular arms that stabilize filaments by binding actin subunits in opposing strands [Lilic et al., 2003]. The crucial role of many ABPs is evident in the diseases caused by mutations in genes encoding ABPs. For example, the Wiskott-Aldrich syndrome, a disease caused by mutations in the WASP gene, affects the immune system, and entails eczema and thrombocytopenia [Binder et al., 2006].

IDPs in the Extracellular Matrix

The ECM surrounds and connects animal cells to form tissue. Consisting of the basement membrane and the interstitial matrix, the ECM provides structural support for the cell and regulates intercellular communication necessary for wound healing, fibrosis and growth [Kim et al., 2011]. The interstitial matrix is composed of polysaccharide gels and fibrous proteins that serve as a buffer against mechanical stress to the ECM and activate growth factors which allow

the regulation of cellular functions as a consequence of extracellular signaling [Kim et al., 2011].

Interestingly, proteins located in the ECM seem to utilize intrinsically disordered domains more frequently than cellular proteins. Especially proteins involved in the organization of the ECM, molecular recognition and cell-matrix adhesion contain long disordered regions [Peysselon et al., 2011]. Accordingly, the amount of amino acid residues predicted to be part of a disordered region was found to reach 57% in members of the collagen superfamily, and 97% in a second family, the small integrin-binding ligand N-linked glycoproteins [Peysselon et al., 2011]. The high levels of intrinsic disorder might be necessary to provide extracellular proteins with the structural flexibility to act as hubs for intracellular, extracellular and membrane-bound interaction partners in a constantly changing environment exposed to mechanical stress [Peysselon et al., 2011].

Conclusions and Perspectives

Structural disorder is everywhere in cytoskeleton, due to the advantages enumerated in the Introduction. Structural disorder is involved in multiple adaptive binding in nucleating polymerization, in regulation by post-translational modifications, in maintaining spacing and connectivity of cytoskeletal elements, in selectively binding multiple cargo proteins and in general regulating the formation, maintenance and rearrangements of the cytoskeleton in countless ways. Because disordered regions can interact in an adaptive process with several proteins, no wonder several of the accessory proteins are involved with more than one cytoskeletal component, that is, they mediate interaction and cross-talk between the three major filamentous systems. Such proteins are usually highly disordered.

Acknowledgments

This work was supported by the Odysseus grant G.0029.12 from Research Foundation Flanders (FWO). The authors declare no conflict of interest.

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