Quality control of cytoskeletal proteins and human disease

Victor F. Lundin1,2, Michel R. Leroux1 and Peter C. Stirling1,3

1 Department of Molecular Biology and Biochemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6, Canada
2 Department of Neurology, Stanford University School of Medicine, Stanford, CA, 94305-5489, USA
3 Michael Smith Laboratories, University of British Columbia, 2185 East Mall, Vancouver, BC, V6T 1Z4, Canada

Actins and tubulins are abundant cytoskeletal proteins that support diverse cellular processes. Owing to the unique properties of these filament-forming proteins, an intricate cellular machinery consisting minimally of the chaperonin CCT, prefoldin, phosducin-like proteins, and tubulin cofactors has evolved to facilitate their biogenesis. More recent evidence also suggests that regulated degradation pathways exist for actin (via TRIM32) and tubulin (via parkin or cofactor E-like). Collectively, these pathways maintain the quality control of cytoskeletal proteins (‘proteostasis’), ensuring the appropriate function of microfilaments and microtubules. Here, we focus on the molecular mechanisms of the quality control of actin and tubulin, and discuss emerging links between cytoskeletal proteostasis and human diseases.

The evolution of cytoskeletal proteins required a novel biogenesis machinery

The cytoskeleton enables processes such as intracellular trafficking and cell division. It was once believed to be a cellular feature that distinguished eukaryotes from prokaryotes. Arguably one of the most rewarding recent findings in biology has been the discovery of a bacterial cytoskeleton composed of proteins homologous to actin and tubulin [1]. Similar to their eukaryotic counterparts, the bacterial actin-related proteins MreB and ParM and the tubulin-related protein FtsZ form filamentous structures implicated in septation, segregation of genetic material, and maintenance of cell shape [1]. Yet, actins and tubulins, two of the most highly conserved eukaryotic proteins, are distinct from their prokaryotic counterparts: they harbor innovative properties critical for eukaryogenesis [2]. For example, actin and tubulin formed microfilaments and microtubules that, together with their associated molecular motors (myosin, kinesin, dynein), were used for phagocytosis—thereby enabling endosymbiosis—and the development of the cilium, a ubiquitous cellular appendage which supports motility and sensory processes [3].

An unfolding realization has been that the inception of eukaryote-like actin and tubulin necessitated the co-evolution of factors that facilitate their efficient folding and assembly [2,4,5]. This cytoskeletal protein biogenesis machinery, absent from prokaryotes, is now recognized to include molecular chaperones that assist folding, namely chaperonin containing tailless complex polypeptide-1 (CCT) and prefoldin (PFD), phosducin-like proteins that regulate CCT functions, and five cofactors critical for assembling α-β-tubulin heterodimers [6–10]. The regulation of the function of actin and tubulin function also demands additional mechanisms unique to eukaryotes, such as post-translational modifications and proteasomal degradation. In this review, we discuss the remarkably complex biogenesis machinery for actins and tubulins, along with recently identified players that appear to assist in their degradation. We also explore evidence that aberrant quality control of cytoskeletal proteins engenders human disease, including developmental and, in particular, neurological disorders.

Autoregulation of cytoskeletal protein synthesis

The concentrations of assembly-competent actin and tubulins must be tightly controlled because they are essential variables affecting cytoskeletal dynamics. Tubulin synthesis in metazoans is also autoregulated via the mechanism that is sensitive to the level of actin monomer concentration [11,12]. Similarly, the 3’ untranslated region of actin mRNA is required for preventing actin overexpression via an incompletely characterized feedback mechanism that is sensitive to the level of actin monomer [13]. Tubulin synthesis in metazoans is also autoregulated at the level of protein synthesis because transgenic expression of α-tubulin reduces production of endogenous α-tubulin without affecting mRNA levels [14]. The total amounts and the ratio of α-tubulin to β-tubulin are important because β-tubulin overexpression induces an increase in the synthesis of endogenous α-tubulin [15]. Interestingly, β-tubulin overexpression in Saccharomyces cerevisiae leads to aberrant microtubule function and slow growth [16], suggesting that multicellular eukaryotes evolved mechanisms for tubulin regulation subsequent to their divergence from yeast.

Biogenesis of cytoskeletal proteins: a significant housekeeping challenge

Actin and tubulin represent a large proportion of the total protein in most cells (although absolute levels can vary with different cellular contexts). Due to their high concentration, propensity to self-associate, and inability to fold...
unaided, newly synthesized actin and tubulin present a significant challenge to the protein biogenesis machinery [2]. The biogenesis of cytoskeletal proteins is additionally complicated because actin and tubulin compete for access to the same limited folding space (i.e. cytosolic chaperonin), and the total outputs of folded actin and tubulin are therefore probably dependent upon each other [2]. The coordinated action of dedicated chaperonin cofactors, including PFD and the phosducin-like proteins, could (at least in part) account for the regulation of chaperonin-mediated cytoskeletal protein folding.

**Eukaryotic cytosolic chaperonin: a unique ability to assist the folding of actin and tubulin**

Newly made polypeptide chains can interact with, and be stabilized by, one or more so-called molecular chaperones during folding from a linear chain of amino acids into a functional protein (Figure 1a) [17]. Several classes of chaperones collectively assist the folding of nascent proteins, refolding of stress-denatured proteins, unfolding of proteins before degradation, and transport of proteins across intracellular membranes [17,18].

One conserved and essential family of molecular chaperones is the chaperonins, which adopt a barrel-like structure with two multimeric stacked rings of ~60-kDa subunits [19]. In eukaryotes, the cytosolic chaperonin is termed CCT or TRiC (tailless complex polypeptide 1 ring complex) and is required for viability in yeast and worms [19,20]. CCT comprises eight related subunits (α, β, γ, δ, ε, ζ, η, θ) present twice in each oligomer, yielding a ~1-MDa complex (Figure 1b). CCT is closely related to the archaeal chaperonin thermosome, and more distantly to the well-characterized bacterial chaperonin GroEL [19]. As with all chaperonins, CCT undergoes ATP-dependent conformational changes during its folding cycle, which facilitate substrate binding, encapsulation, and release [21].

CCT is crucial for the biogenesis of actin and tubulin, an activity lacking in prokaryotic chaperonins [19]. Based on their abundance, actin and tubulin probably occupy a substantial proportion of CCT complexes at any given time [22]. Although its known substrate repertoire has expanded considerably in recent years, CCT is considered to have a more specific binding profile than bacterial GroEL [22,23]. Substrate proteins seem to contact multiple specific subunits while bound to CCT so that multiple binding sites provide combinatorial specificity [24,25]. This property has been demonstrated for its cytoskeletal protein substrates, whereby non-native actin contacts two subunits and tubulin interacts with five subunits [24]. The specificity of these interactions imply that CCT co-evolved with actin and tubulin during emergence of the ancestral eukaryote; this hypothesis is now supported by direct biochemical evidence [2,4].

Aside from actin and tubulin, other CCT substrates have key roles in progression of the cell cycle, including the anaphase-promoting complex regulators CDH1 and CDC20 [19]. In this light, the association of CCT with colorectal cancer is particularly interesting and could suggest a unique requirement for chaperonin activity in these abnormal cells (Box 1) [26]. Furthermore, insulin signaling and nutrient signaling seem to converge on the phosphorylation of CCTβ Ser260, a modification required for the proliferation of normal cells [27]. Ser260 is located at the tip of the helical protrusion which forms the functionally essential lid of CCT (Figure 1b), suggesting that modulation of CCT-mediated folding of cytoskeletal proteins could be an important regulatory step in multiple biochemical pathways. Given the specialized requirement for CCT in the biogenesis of actin and tubulin and its broader role in assisting the folding and assembly of numerous other proteins, including centractin and gamma-tubulin, it is perhaps unsurprising that CCT function in vivo is regulated by several dedicated cofactors, including PFD and phosducin-like proteins.

**PFD: a jellyfish-shaped molecular chaperone required for stabilization of nascent cytoskeletal proteins**

PFD is a dedicated CCT co-chaperone for the biogenesis of actin and tubulin (Figure 1b) [28,29]. Eukaryotic and archaeal PFD form jellyfish-shaped hexameric complexes consisting of two α-type, and four β-type subunits, with six unique subunits in eukaryotes and typically single α- and β-subunits in archaea [29,30]. The six tentacle-like PFD subunits form a rectangular cavity which binds the partially

**Box 1. Cytoskeletal biogenesis and cancer**

The importance of the cytoskeleton in cancer has long been appreciated. For example, actin remodeling can facilitate invasive growth of tumors [73], and the rapid mitoses of proliferating cancer cells probably require rapid turnover of the mitotic spindle [74]. Conversely, disrupted function of the mitotic spindle can lead to chromosome instability (CIN), a hallmark of most solid tumors [75]. Thus, the cytoskeletal biogenesis machinery could prevent oncogenesis by maintaining normal function of the mitotic spindle or facilitate progression of rapidly growing tumors; there is considerably more evidence for the latter hypothesis. PFD overexpression has been observed in many tumor types including pancreatic cancer and neuroblastoma [76,77], and CCT overexpression has been observed in liver cancer and in the late stages of colon cancer [26,78]. Taken together, these findings suggest that PFD and CCT are permissive for uncontrolled proliferation and, given the importance of microtubules for cell proliferation, the link between CCT/PFD and cancer probably relates to tubulin biogenesis as opposed to an unidentified folding substrate. Many CIN tumors are sensitized to spindle dysfunction, so some anti-tumor drugs are specific tubulin-binding agents (e.g. vinblastine, paclitaxel/taxol) which promote stabilization or disassembly of microtubules to arrest cell division and induce apoptosis [79].

It is clear that the cytoskeletal proteostasis machinery will be engaged in rapidly growing tumor cells and in their response to anti-tubulin chemotherapeutics. In a novel anti-mitotic approach, a recent study showed that disrupting the CCT-β-tubulin interaction with a small molecule induces apoptosis even in cancer cells resistant to other tubulin-binding chemotherapies [79]. CCT has another link to cancer whereby its interaction with the von Hippel–Lindau (VHL) tumor suppressor protein is essential for assembly of the VHL E3 ubiquitin ligase complex; tumor-inducing mutations in VHL specifically disrupt CCT binding [80]. Bisphosphonates, which are used to treat cancer-induced bone loss (and which are the subject of clinical trials against breast cancer due to their effects on the migration, invasion and proliferation of cancer cells) trigger a strong upregulation of TCB and prominent disruption of the microtubule cytoskeleton [81]. Drugs targeting tubulin folding cofactors could be beneficial because they would be tubulin-specific and could have anti-proliferative and anti-metastatic effects.
Figure 1. Cellular proteostasis of actins and tubulins. Briefly, a cytoskeletal protein emerging from the ribosome (a) (actin, α-tubulin or β-tubulin) is bound by PFD and delivered to CCT, or bound by CCT directly (b). CCT-mediated folding, regulated by PhLPs and PFD, releases native or near-native actin (red) and near-native, GTP-bound α-tubulin or β-tubulin (dark or light-green, respectively). Native actin released from CCT (or possibly via an interaction with cyclase-associated protein (CAP)) can assemble into actin filaments (c-iv). This process is regulated by nucleotide binding, hydrolysis and exchange in actin and by a host of additional accessory factors (not shown). Actin monomers could be targets of E3 ubiquitin ligases such as TRIM32 which can mediate actin polyubiquitylation, thereby triggering proteasomal degradation (d-iii). After release from CCT, α-tubulin and β-tubulin are bound by TBCB and TBCA, respectively, before transfer to TBCE and TBCD (where possible, relevant domain names are indicated beside the first appearance of each cofactor). The protein complex consisting of α-tubulin, β-tubulin, TBCE and TBCD (c-i) recruits TBCC, which stimulates β-tubulin GTP hydrolysis and the subsequent release of the native α/β-tubulin heterodimer. Native tubulin heterodimers (c-ii) can exchange GDP for GTP in the β-subunit and assemble into microtubules, after which an array of binding proteins and modifying enzymes further regulate microtubule function (not shown). Native heterodimers can also be sequestered by proteins such as stathmin, or previously existing post-translation modifications can be reversed (not shown). Finally, native tubulin heterodimers can be bound by TBCB, TBCE, COEL and TBCD, in cooperation with RP2 or ARL2, as part of several proposed α-tubulin and β-tubulin quality control mechanisms (c-iii). At least two pathways can lead to recycling or degradation of α/β-tubulin. Heterodimers can be dissociated by TBCB, TBCD, TBCE and COEL and each tubulin subunit can be targeted by unknown E3 ubiquitin ligases for proteasomal degradation (d-ii) or some other form of regulated proteolysis (not shown). Alternatively, intact heterodimers can be targeted for proteasomal degradation by E3 ubiquitin ligases such as parkin (d-i). For simplicity, the partially characterized autoregulatory feedback mechanisms operating to buffer the systems against dramatic shifts in protein concentration are not shown. Except for the ribosome, protein crystal structures are shown where applicable. The CCT structure shown is based on the archaeal thermosome. Protein structures are colored as follows: blue, sites in molecular chaperones probably involved in cytoskeletal protein interactions; cyan, site of CCT phosphorylation at S260; and gray, nucleotide.
folded chains of actin and tubulin emerging from the ribosome before delivering them to CCT, probably by docking and direct substrate hand-off [5,17,29]. Electron microscopy (EM) of PFD–actin complexes suggests a ‘hand-in-glove’ fit consistent with the co-evolution of specific binding sites for actin [2,5,29], similar to the situation for CCT. In addition to its function of substrate delivery, PFD might also enhance the efficiency of actin and tubulin folding by acting as a transient ‘cap’ for CCT that returns partially folded substrate molecules to the chaperonin for another round of assisted folding [31].

Yeast cells lacking PFD function fold actin and tubulin more slowly than wild-type cells, and accumulate reduced steady-state concentrations of each protein in vivo (i.e. 50–60% of wild-type for actin and α-tubulin, and 90% of wild-type for β-tubulin) [6,28,31]. Thus, yeast PFD mutants are viable because they produce sufficient concentrations of native actin and tubulin via slow, PFD-independent pathways, perhaps by direct binding of nascent polypeptides by CCT. By contrast, the loss of PFD in Caenorhabditis elegans is embryonic lethal due primarily to an increased demand for tubulin production in mitotic cells [20]. These results are consistent with the predominately tubulin-related phenotypes caused by reducing CCT function in cultured mammalian cells [32]. Pfd1 knockout mice are (perhaps surprisingly) viable, but only for five weeks; they display various phenotypes ascribed to dysfunction of cytoskeletal proteins, including ciliary dyskinesia (loss of motility in the microtubule-based organelle), neuronal loss, neuromuscular defects, and defective development of lymphocytes [33]. Overall, data on PFD function in vivo suggests that, as is probably the case with CCT, the substrate profile and requirement for PFD function might differ according to its cellular context.

Similar to PFD, the eukaryotic and bacterial heat shock protein (HSP) 70 molecular chaperones are widely believed to participate in de novo folding pathways in conjunction with their cognate chaperonins [17]. Notably, a recent EM reconstruction suggests that, in eukaryotes, the mechanism involves a physical interaction between HSP70 and CCT near the opening of the chaperonin cavity, indicative of a direct hand-off of substrates [34]. It is unclear, if under certain circumstances, nascent actin and tubulin proteins make use of the otherwise promiscuous HSP70 chaperone.

Phosducin-like proteins: regulators of the folding of actin and tubulin in association with CCT

The phosducin-like family of proteins (PhLPs) are thioredoxin domain-containing proteins with homology to phosducin, a regulator of retinal G-protein signaling [35]. All three family members (PhLP1, PhLP2, PhLP3) have been identified as CCT-binding proteins [7,8,36,37]. PhLP1 seems to be primarily involved in the assembly of heterotrimeric G-proteins by CCT, whereas PhLP2 and PhLP3 participate in the biogenesis of cytoskeletal proteins [7,8,36,37].

It is probable that all PhLPs span the entrance of the CCT substrate-binding cavity, forming a ternary complex with substrate-bound CCT (Figure 1b) [7,8,38]. Proteomic analyses of yeast CCT-binding proteins suggest that this ternary complex is ATP-labile [22]. Asymmetric ternary complexes with substrate and CCT have been detected for all PhLPs, but it is unknown whether PhLP occupies the cis- or trans-chaperonin ring with respect to substrate [7,8,37]. In vitro, an excess of mammalian PhLP2 or PhLP3 inhibits CCT-mediated folding of actin and tubulin, probably via reduced activity of CCT ATPase [7,8]. Conversely, yeast PhLP2 stimulates actin folding by purified yeast CCT in vitro [9]. These authors showed that specific amino acids of mammalian PhLP2, absent from yeast PhLP2, define its inhibitory effect and suggest that additional regulation has evolved in higher eukaryotes. PhLP1–CCT-mediated G protein assembly is regulated by PhLP1 phosphorylation; perhaps PhLP2- or PhLP3-mediated biogenesis of cytoskeletal proteins could be regulated in this manner in certain systems [37].

Disruption of PhLP3 in yeast, C. elegans and Arabidopsis thaliana primarily interferes with normal tubulin biogenesis/microtubule function, although some actin cytoskeletal phenotypes have been observed in yeast [6,7,39,40]. PhLP2 disruption is lethal in all organisms tested, and temperature-sensitive alleles in yeast reveal severe actin cytoskeletal defects along with minor cell-cycle and tubulin defects [8,35]. Taken together, these data suggest the potential specificity of PhLP3 for tubulin biogenesis and PhLP2 for actin biogenesis [9]. It is also plausible that other CCT substrates might be influenced by PhLPs; the cell-cycle phenotypes observed in Plp2 (PhLP2) mutant yeast are consistent with this notion [8]. In dissecting the molecular mechanism by which PFD and PhLPs affect CCT function, determining if these cofactors control the selectivity of chaperonin binding under different cellular conditions (e.g. favoring the folding of actin, tubulin or other substrates) will be interesting.

Tubulin folding cofactors

In general, it is believed that actin is released from CCT in a native, assembly-competent state, but cyclase associated protein (CAP) might also interact with and stabilize near-native or unstable forms of actin in close association with the chaperonin (Figure 1b) [41]. By contrast, functional tubulin is an obligate α–β heterodimer, and has evolved a folding pathway linked to dimer assembly (Figure 1c) [10]. After release from CCT in vivo, quasi-native β-tubulin monomers can bind to tubulin folding cofactor A (TBCA), which acts as a sink for unassembled β-tubulin, yet is dispensable for heterodimer assembly from denatured proteins in vitro [42]. To progress along the assembly pathway, β-tubulin interacts with cofactor D (TBCD). A parallel system exists for α-tubulin in which, after chaperonin release, it binds cofactor B (TBCB) and is subsequently transferred to cofactor E (TBCe) for further processing. TBCB facilitates in vitro heterodimer assembly, and failure to degrade TBCB appropriately has been linked to the neurological disease giant axonal neuropathy, which is associated with reduced microtubule density (Table 1, Box 2) [42,43]. The essential feature of the tubulin folding cofactor system, which has been reconstituted in vitro, is that the β-tubulin–TBCD and α-tubulin–TBCe complexes associate to form a stable supercomplex (Figure 1c-i) which, in the presence of cofactor C (TBCc), stimulates GTP hydrolysis in
The cilium is a microtubule-based organelle implicated in multiple human disorders, including obesity, polycystic kidney disease and blindness [87]. Interestingly, at least two classes of ciliopathy-associated proteins could influence microtubule biogenesis. Retinitis pigmentosa 2 protein (RP2) is a paralog of TBCC and both associated proteins could influence microtubule proteostasis from excess TBCB, probably disrupting tubulin proteostasis.

Box 2. Heritable diseases of cytoskeletal homeostasis pathways

Point mutations in actin and tubulin isoforms are associated with heritable diseases and, in some cases, the pathological mechanism involves disrupted interactions with the cytoskeletal proteostasis machinery. For example, mutations in cardiac α-actin (which cause dilated or hypertrophic cardiomyopathy) interrupt the normal interaction with CCT, leading to slow folding, aggregation and an inability to incorporate into actin filaments [82]. Similarly, certain α-tubulin and β-tubulin mutations causing lissencephaly/pachygyria and asymmetrical polymicrogyria, respectively, disrupt tubulin association with the post-CCT folding cofactor TBCA in the case of β-tubulin, and TBCB in the case of α-tubulin (Table 1) [83–85].

Inappropriate expression or mutation of cytoskeletal proteostasis genes is also associated with human diseases (Table 1). Timely degradation of TBCB in neurons is regulated by the E3 ubiquitin ligase gigaxonin; mutations in this ligase cause the neurodegenerative disease giant axonal neuropathy (GAN) [43]. TBCB accumulation results in microtubule dysfunction and might contribute to neurotoxicity in GAN [43]. Similarly, mutation of human CCT5 is linked to neurodegeneration in the rare disorder mutilating sensory neuropathy with spastic paraplegia [86]. TBCE mutations are linked to the severe developmental disorder hypoparathyroidism, mental retardation, facial dysmorphism and AR-Kenny–Caffey syndrome, which is associated with reduced microtubule density and abnormal microtubule polarity in cells cultured from patients [47].

The cilium is a microtubule-based organelle implicated in multiple human disorders, including obesity, polycystic kidney disease and blindness [87]. Interestingly, at least two classes of ciliopathy-associated proteins could influence microtubule biogenesis. Retinitis pigmentosa 2 protein (RP2) is a paralog of TBCC and both associated proteins could influence microtubule proteostasis from excess TBCB, probably disrupting tubulin proteostasis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein function</th>
<th>Associated disease</th>
<th>Molecular etiology</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tubulin (TUBA1)</td>
<td>Constituent of microtubules</td>
<td>Pachygyria</td>
<td>Disrupted binding to CCT, TBCB and GTP</td>
<td>85</td>
</tr>
<tr>
<td>α-tubulin (TUBA3)</td>
<td>Constituent of microtubules</td>
<td>Lissencephaly</td>
<td>Disrupted biogenesis of tubulin or disrupted microtubule function</td>
<td>84</td>
</tr>
<tr>
<td>β-tubulin (TUBB2B)</td>
<td>Constituent of microtubules</td>
<td>Asymmetrical polymicrogyria</td>
<td>Disrupted TBCA/TBCD binding, reduced polymerization</td>
<td>83</td>
</tr>
<tr>
<td>α-actin (ACTC)</td>
<td>Constituent of microfilaments</td>
<td>Dilated or hypertrophic cardiomyopathy</td>
<td>Disrupted binding of cardiac α-actin to CCT</td>
<td>82</td>
</tr>
<tr>
<td>CCT5</td>
<td>Protein folding chaperonin</td>
<td>AR*-mutating sensory neuropathy with spastic paraplegia</td>
<td>Unknown</td>
<td>86</td>
</tr>
<tr>
<td>CCT4</td>
<td>Protein folding chaperonin</td>
<td>Hereditary sensory neuropathyb</td>
<td>Unknown</td>
<td>98</td>
</tr>
<tr>
<td>TBCE</td>
<td>α-tubulin folding, heterodimer assembly and disassembly</td>
<td>Hypoparathyroidism-retardation-dysmorphism and AR-Kenny–Caffey syndrome</td>
<td>Inefficient folding and assembly of tubulin leading to cellular microtubule defects</td>
<td>47,48</td>
</tr>
<tr>
<td>Gigaxonin (GAN1)</td>
<td>E3 ubiquitin ligase for TBCBc</td>
<td>Giant axonal neuropathy</td>
<td>Microtubule dysfunction with contribution from excess TBCB, probably disrupting tubulin proteostasis</td>
<td>43</td>
</tr>
<tr>
<td>Parkin (PARK2)</td>
<td>E3 ubiquitin ligase for α/-β-tubulin*</td>
<td>AR-juvenile PD</td>
<td>Unknown, with possible contributions from disrupted ERAD of Hsp70-CHIP substrates and disrupted tubulin degradation</td>
<td>61,62</td>
</tr>
<tr>
<td>RP2</td>
<td>Tubulin GTPase-activating protein</td>
<td>Retinitis pigmentosa</td>
<td>Disrupted maintenance of connecting cilium, with possible contribution from ciliary/basal body tubulin quality control</td>
<td>88,89</td>
</tr>
<tr>
<td>BBS6</td>
<td>CCT-like protein of unknown function</td>
<td>Bardet-Biedl Syndrome (BBS)</td>
<td>Disrupted ciliogenesis and centrosomal/basal body function, with possible contribution from tubulin quality control</td>
<td>90</td>
</tr>
<tr>
<td>BBS10</td>
<td>CCT-like protein of unknown function</td>
<td>BBS</td>
<td>Disrupted ciliogenesis, with possible contribution from disrupted tubulin quality control</td>
<td>99</td>
</tr>
<tr>
<td>BBS12</td>
<td>CCT-like protein of unknown function</td>
<td>BBS</td>
<td>Disrupted ciliogenesis, with possible contribution from disrupted tubulin quality control</td>
<td>100</td>
</tr>
<tr>
<td>TRIM32 (BBS11)</td>
<td>E3 ubiquitin ligase for actinb</td>
<td>BBS, limb girdle muscular dystrophy 2H, myopathyb</td>
<td>Unknown, possible contribution from defective actin degradation</td>
<td>70,71,72</td>
</tr>
<tr>
<td>ELP1</td>
<td>Scaffolding component of Elongator</td>
<td>Familial dysautonomia</td>
<td>Partially known, with contribution from disrupted tubulin acetylation</td>
<td>51</td>
</tr>
<tr>
<td>ELP3</td>
<td>Acetylase component of Elongator</td>
<td>Amyotrophic lateral sclerosis</td>
<td>Unknown, with possible contribution from disrupted tubulin acetylation</td>
<td>51,52</td>
</tr>
</tbody>
</table>

*AR = autosomal recessive; *Mouse disease models only; *E3 ubiquitin ligases might have other disease-relevant substrates.
β-tubulin and subsequent release of the native α-β-tubulin heterodimer (Figure 1c-ii).

The complexity of this pathway suggests that tubulin biogenesis is particularly error-prone or that it is co-regulated with other processes. In this context, TBCC, TBCD and TBCE act as a GTPase-activating complex for heterodimeric β-tubulin, thus potentially identifying aberrant GTP-locked heterodimers (Figure 1c-iii) [44]. Furthermore, TBCD and TBCE can dissociate native heterodimers, which could facilitate changes in the tubulin isotype composition of the heterodimer pool [42,45]. The small GTPase ADP-ribosylation factor-like 2 (ARL2) negatively regulates the tubulin-GAP and heterodimer disassembly activities of TBCD, but little is known about the signals that impinge upon ARL2 function in vivo (Figure 1c-iii) [45]. Recent evidence indicates that TBCB and TBCE form a dimer which dissociates native tubulin heterodimers and, because TBCB and TBCE contain ubiquitin-like (UBL) domains that typically mediate interactions with the ubiquitin–proteasome system (UPS), might also regulate the degradation of α-tubulin [46]. TBCE mutations have been linked to the developmental disorder hypoparathyroidism, mental retardation, facial dysmorphism (HRD) (Table 1, Box 2) [47]. Subsequent work has identified cryptic translation sites in mutant TBCE which produce reduced levels of functional protein, rescuing the lethality expected for loss of TBCE function [48]. This study also suggested a mechanism of pathogenesis linked to a reduced capacity for de novo heterodimer folding causing non-lethal cellular microtubule defects [48].

Post-translational modification (PTM) of cytoskeletal proteins

In addition to folding and assembly, covalent modification is often an integral part of the biogenesis pathway leading to functional proteins. These PTMs can affect folding or act on native proteins as reversible ‘switches’ that regulate activity (Box 3). In the case of actin, there is little or no evidence for the latter, whereas tubulin is subject to a host of reversible modifications, including acetylation, detyrosination and glutamylation [49]. In general, it is believed that, whereas tubulin modifications occur on microtubules, the substrate for the reversal of these modifications is the free tubulin heterodimer, but this hypothesis has not been extensively tested. Some of the tubulin-modifying enzymes might compete or cooperate with other factors involved in regulating tubulin function. For example, the tubulin deacetylase HDAC6 interacts with several microtubule-binding proteins, including the +end tracking protein EB1 [50]. Tubulin PTMs have received significant attention. Recent evidence suggesting that tubulin acetylation might be involved in the human neurodegenerative disease amyotrophic lateral sclerosis (ALS) should increase investigative efforts in this area [51,52]. Although there is no evidence to suggest a direct link between tubulin modification and the quality control machinery, the presence of at least one irreversible tubulin modification (Δ2-tubulin, lacking the C-terminal Glu-Tyr dipeptide) suggests that control of the covalently modified tubulin composition might also require regulated degradation [49].

Degradation of cytoskeletal proteins

Protein degradation through the UPS, lysosomal degradation or another proteolytic process controls the turnover rates and steady-state concentrations of all proteins in the cell [53]. Regulated degradation of proteins is also part of the quality control process or ‘proteostasis’ whereby damaged or misfolded proteins that cannot be refolded by molecular chaperones are removed by proteolysis [18]. In contrast to the pathway of biogenesis of cytoskeletal proteins, the compensating process of actin and tubulin degradation has not been thoroughly investigated, but recent studies indicate that it is relevant to human disease (Table 1, Box 4).

Tubulin degradation

Although α-β-tubulin heterodimers are very stable, the mean half-life of tubulin does not reflect the fact that tubulin is partitioned into multiple states that could have

---

**Box 3. Covalent modification of cytoskeletal proteins**

Covalent tubulin modifications (CTMs) were first observed several decades ago and our understanding of their importance in microtubule regulation has improved significantly since then [49]. The current view is that CTMs alter the surface properties of the microtubule lattice. Elaborate CTM patterns have been observed along axonal microtubules and subfibers of ciliary axonemes [49,91]. CTMs are differentially recognized by microtubule-associated proteins (MAPs) such as kinesin whose motility is increased by acetylation of α-tubulin Lys40 [92]. Tubulin hyperacetylation via pharmacological inhibition of HDAC6 disrupts polarized trafficking in neurons, leading to mislocalization of neurite-specific cargoes [92]. The recent discovery of the α-tubulin acetylase elongator subunit 3 (ELP3) will probably initiate studies probing tubulin acetylation and microtubule function [51] because ELP3 mutations have been linked to the neurodegenerative disease amyotrophic lateral sclerosis [52].

Acetylation and detyrosination of tubulin are routinely used as markers for stable microtubules although neither affects microtubule dynamics in vitro [49]. The elusive relationship between tubulin tyrosination and microtubule stability was recently clarified because the microtubule depolymerizing kinesins MCAK and Kif2A have a lower affinity for detyrosinated microtubules [93]. Similarly, the tubulin tyrosination cycle mediates the binding of CAP-Gly proteins to microtubule +ends, which helps to explain the essential role of tubulin tyrosine ligase in the motility and morphology of neuronal cells [49].

CTMs mediate interactions with MAPs, so the functional consequences of a given modification can vary according to the cellular context. For example, in the unicellular ciliate *Tetrahymena thermophila*, tubulin hyperglutamylation, via overexpression of tubulin tyrosine ligase-like 6A (TTLL6A), results in excessively stable cytosolic microtubules but causes unstable microtubules in the ciliary axoneme [94].

Covalent modifications of actin have not been extensively studied, and the observed N-methylhistidine at residue 73 and acetylation of the N-terminus are thought to be part of the protein maturation process [95]. Several tyrosine residues on actin are targets of oncogenic kinases, but the effects of these modifications on actin function have not been determined [96]. Recent evidence supports the existence of an O-linked N-acetylglicosaminyltransferase (O-GlcNAC) site between residues 318 and 324 and, although the physiological significance of this modification is unknown, it might begin to explain how O-GlcNAC-transferase inhibition affects the cell cycle [97].
The production of appropriately folded actin and tubulin subunits requires a network of specific molecular chaperones acting in a coordinated fashion. The abundance and complex topological folding requirements of actin and tubulin have necessitated this system which, like actin and tubulin, is only found in eukaryotes. The timely folding of actin and tubulin is critically important for cytoskeletal function because it requires that a pool of native subunits is poised for recruitment into cytoskeletal structures. Defects in the folding of actin or tubulin are typically lethal in metazoans, even hypomorphic mutations in chaperones required for actin/tubulin biogenesis lead to several debilitating human diseases (Table 1, Box 2) [47,86,98]. Structural and mechanistic descriptions of CCT, PFD, the tubulin folding cofactors and PhLPs remain incomplete. One important future direction will be to understand the cellular signals that impinge upon the biogenesis machinery to regulate the output of cytoskeletal building blocks. For example, CCT is phosphorylated in vivo, but the effects of this modification on the folding of actin and tubulin remain unknown [27]. Mounting evidence suggests that the biogenesis machinery does not act as a constitutive folding pathway, but that some factors might also act in a dynamic way to mediate protein function and quality control. Thus, a novel paradigm emerging for the regulation of cytoskeletal building blocks is that the biogenesis and degradation of proteins are both important for normal function, and that there could be cross-talk between these pathways. This idea is exemplified by the different effects of TBCB, TBCE, COEL and parkin on the function and stability of tubulin [46,59,62]. Further research on the regulated degradation of actin and tubulin should help us to better understand how biogenesis, modification and degradation pathways affect the essential functions of the eukaryotic cytoskeleton.

very different turnover rates (i.e. chaperone–monomer complexes; GTP-bound, GDP-bound and covalently modified heterodimers; dynamic and stable microtubules). Importantly, tubulin can be rapidly degraded in response to microtubule-destabilizing drugs such as colcemid, which increases the concentration of soluble tubulin [54]. In the absence of normal PFD or CCT function, tubulin cannot fold efficiently and non-native tubulin is removed by proteolysis [20,32]. Treatment of HeLa cells with the proteasome inhibitor MG132 indicated that α-tubulin and β-tubulin are ubiquitylated [55]. Even tubulin in otherwise stable axonemal microtubules appears to be targeted to the proteasome in response to flagellar shortening [56]. TBCE overexpression has been reported to stimulate α-tubulin proteolysis [57]. This finding contradicts other results [46] and could indicate cell-type specific variations in the tubulin regulatory machinery (12). Although the specific players involved might vary, the capacity for regulated tubulin degradation is likely to be ubiquitous. For instance, tubulin from various human cell types is targeted by cancer-preventive isothiocyanates which covalently modify tubulins and induce conformational changes that trigger proteasome-dependent tubulin degradation [58].

Cofactor E-like
Tubulin folding cofactor E-like (COEL), a novel protein identified by its sequence similarity to TBCE, was recently characterized as a tubulin-destabilizing protein [59]. TBCE and COEL contain leucine-rich repeat and C-terminal UBL domains. Unlike TBCE, COEL lacks the N-terminal CAP-Gly domain, which has been implicated in tubulin binding [60]. Despite this absence, COEL displaces β-tubulin from heterodimers in vitro and remains bound to α-tubulin. Cells depleted of human COEL by siRNA possess an excess of stable microtubules, whereas COEL overexpression causes microtubule disassembly and degradation of proteasomal degradation of α-tubulin [59]. COEL lacks the domains required for ubiquitylation or proteolysis, so it must recruit other proteins to degrade α-tubulin. Interestingly, the stimulation of tubulin degradation by COEL overexpression is counteracted by stathmin, a negative regulator of microtubule dynamics that sequesters tubulin heterodimers [57]. One could envision COEL as part of a concerted quality control mechanism linking the biogenesis and degradation of α-tubulin. Such a mechanism could be important for the:

(i) removal of misfolded tubulin occurring during its complex folding pathway (particularly during rapid synthesis or stress); (ii) robust regulation of steady-state tubulin concentration under diverse conditions; (iii) dynamic control of the tubulin isotype or covalently modified tubulin concentrations.

Parkin
One factor directly implicated in tubulin degradation is the RING-family E3 ubiquitin-protein ligase parkin, which is mutated in patients with autosomal recessive juvenile Parkinson disease (PD; Table 1). Parkin ubiquitylates stress-denatured proteins in cooperation with carboxyl terminus of HSP70-interacting protein (CHIP) and HSP70 [61]. Additionally, convincing evidence suggests that parkin is microtubule-associated and promotes the ubiquitylation and proteasomal degradation of α-tubulin and β-tubulin (Figure 1) [62]. The localization of parkin along microtubules is consistent with its role in ubiquitylating proteins exported from the endoplasmic reticulum (ER), misfolded proteins transported to the perinuclear aggresome, and damaged tubulin subunits [63]. Although a role for tubulin accumulation in PD is speculative, accumulation of insoluble parkin and α-tubulin has been observed in cells overexpressing mutant α-synuclein (the toxic inclusion-forming protein in PD) and in patients with Lewy body disease [64]. Given the ubiquitous and essential function of tubulin, the finding that, in several systems, parkin mutations lack obvious consequences suggest that there could be redundant pathways for tubulin degradation [61,65].

Degradation of β-tubulin
β-tubulin undergoes cycles of GTP-hydrolysis and nucleotide exchange whereas α-tubulin does not. Hence, regulated turnover of β-tubulin could differ from that of α-tubulin if, for example, the enzymatic activity of β-tubulin made it more susceptible to oxidative damage or misfolding than α-tubulin. In S. cerevisiae, β-tubulin overexpression is toxic, whereas α-tubulin overexpression is tolerated [16]. This effect is not observed in mammalian cells due to the autoregulation (and perhaps degradation) of β-tubulin. Given the role of TBCD and ARL2 in regulating β-tubulin assembly, disassembly and GTP hydrolysis, it is tempting to speculate that these proteins might also be
involved in regulating β-tubulin degradation. TBCD is particularly interesting because it can dissociate tubulin heterodimers and contains an Armadillo repeat domain, a motif that was recently implicated in mediating substrate ubiquitylation [66,67].

Actin degradation
The quality control of actin appears to differ fundamentally from that of tubulin. If the activity of CCT or PFD is inhibited in metazoan cells, the total concentration of tubulin is strongly reduced whereas the actin concentration remains relatively unchanged, despite the fact that non-native actin is entering the cytosol [20,32]. The need to remove misfolded β-actin therefore might not be as tightly regulated as for tubulins. Nevertheless, actin degradation seems to be important under certain conditions. For example, cardiac-specific α-actin is degraded by the proteasome after ischemic oxidative damage, but this seemingly occurs without the accumulation of ubiquitylated intermediates [68]. There is also evidence that pharmacological inhibition of muscle contraction in cardiomyocytes leads to α-actin degradation mediated (at least in part) by the lysosome [69].

Recently, perhaps more broadly applicable observations of actin degradation were made regarding the RING-family E3 ubiquitin ligase tripartite motif-containing 32 (TRIM32), which binds myosin in skeletal muscle and ubiquitylates α-actin in vitro (Figure 1) [70]. Ectopic expression of TRIM32 in human embryonic kidney cells leads to a reduced level of cytoplasmic β-actin, consistent with a general role in regulating actin degradation [70]. TRIM32 mutations have been implicated in two human diseases (the ciliary disorder Bardet–Biedl syndrome and muscular dystrophy), but the precise role of TRIM32 in the molecular etiology of these diseases is unknown (Table 1) [71,72]. We anticipate that further studies will probe actin proteostasis because its abundance in many cell types represents a significant potential source of proteotoxicity.

Concluding remarks
Although a dedicated folding system for actin and tubulin was discovered nearly two decades ago, our understanding of the complex cytoskeletal quality control pathway continues to expand (Figure 1). Many questions in this field remain unanswered, particularly surrounding the degradation of cytoskeletal proteins (Box 4). Elucidating the details of these pathways will continue to shed light on the importance of the cytoskeleton in biology. It has also become clear that understanding the quality control of cytoskeletal proteins will enhance our knowledge of debilitating human diseases, including cancer, developmental diseases, and neurodegenerative diseases (Box 1 and Box 2).

Disclosure Statement
The authors declare no conflicts of interest.

Note added in proof
An important study has now established that the chaperonin-like proteins BBS6, BBS10 and BBS12 co-assemble with CCT to assist in the folding/assembly of the BBSome—a multi-subunit complex containing several proteins implicated in Bardet-Biedl syndrome [101].

Acknowledgements
We apologize to those colleagues whose work we were unable to cite due to limited space and thank Michael Silverman for critical reading of the manuscript. M.R.L. acknowledges funding from the Canadian Institutes of Health Research (CIHR; grant MOP-84523), as well as a Senior Scholar Award from the Michael Smith Foundation for Health Research (MSFHR) and a New Investigator award from CIHR. P.C.S. is a Research Fellow of The Terry Fox Foundation (award number 700044) and acknowledges the support of MSFHR and Natural Sciences and Engineering Research Council of Canada post-doctoral fellowships. V.F.L. is supported by a Human Frontier Science Program (HFSP) post-doctoral fellowship.

References
5 Martin-Benito, J. et al. (2007) Divergent substrate-binding mechanisms reveal an evolutionary specialization of eukaryotic prefoldin compared to its archaean counterpart. Structure 15, 101–110
74 Huang, H-C. et al. (2009) Evidence that mitotic exit is a better cancer therapeutic target than spindle assembly. *Cancer Cell* 16, 347–358
77 Cimmino, F. et al. (2007) Comparative proteomic expression profile in all-trans retinoic acid differentiated neuroblastoma cell line. *J. Proteome Res.* 6, 2550–2564