Review

Capturing protein tails by CAP-Gly domains

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Cytoskeleton-associated protein-glycine-rich (CAP-Gly) domains are protein-interaction modules implicated in important cellular processes and in hereditary human diseases. A prominent function of CAP-Gly domains is to bind to C-terminal EEY/F-COO$^-$ sequence motifs present in α-tubulin and in some microtubule-associated protein tails; however, CAP-Gly domains also interact with other structural elements including end-binding homology domains, zinc-finger motifs and proline-rich sequences. Recent findings unravelled the link between tubulin tyrosination and CAP-Gly-protein recruitment to microtubules. They further provided a molecular basis for understanding the role of CAP-Gly domains in controlling dynamic cellular processes including the tracking and regulation of microtubule ends. It is becoming increasingly clear that CAP-Gly domains are also involved in coordinating complex and diverse aspects of cell architecture and signalling.

Overview of CAP-Gly proteins

The cytoskeleton-associated protein-glycine-rich (CAP-Gly; Pfam [http://pfam.sanger.ac.uk] entry PF01302) domain, recognized for the first time in 1993 [1], is a specialized protein module of ~80 amino acids that is highly conserved in all eukaryotes. CAP-Gly domains have important roles in functionally diverse proteins, including cytoplasmic linker proteins (CLIPs), the large subunit of the dynactin complex p150^{glued}, tubulin-folding cofactors B and E (TBCB and TBCE), the centrosome-associated protein 350 (CAP350), the kinesin (see Glossary) protein KIF13B (also known as guanylate-kinase-associated kinesin [GAKIN]) and the familial cylindromatosis tumour suppressor CYLD. These multi-domain and/or multi-subunit CAP-Gly proteins (Figure 1) are implicated in important cellular processes including chromosome segregation, establishment and maintenance of cell polarity, intracellular organelle and vesicle transport, cell migration, intracellular signalling and tumourigenesis (for reviews, see Refs [2–6]).

CAP-Gly domains are found in single or multiple copies and are primarily involved in protein interactions and the formation of protein networks [2–6]. Recent studies have provided important advances in understanding the structure–function relationship of CAP-Gly-domain interactions with their binding partners, in particular microtubules and microtubule-plus-end-tracking proteins (+TIPs; Box 1). In addition to regulating microtubule organization and dynamics, CAP-Gly domains are also involved in coordinating complex cellular functions, such as distribution of membrane organelles and intracellular signalling [2–6].

Here, we summarize the emerging principles underlying CAP-Gly-protein functions by focusing on the specific roles of their CAP-Gly domains. We review the structural

Glossary

Ankyrin repeat: a 33-residue protein motif consisting of two α-helices separated by loops. Ankyrin repeats mediate protein–protein interactions and are among the most common structural motifs in proteins.
Catastrophes: transitions from microtubule growth to shortening.
Centrosome: an organelle that serves as the main microtubule organizing centre of the animal cell in addition to a regulator of cell-cycle progression.
Coiled-coil domain: a protein structural motif that mediates subunit oligomerization. Coiled coils contain several (between two and five) α-helices that twist around each other to form a supercoil.
C-terminal EEY/F sequence motif: a highly specific and conserved sequence motif found at the C-termini of α-tubulin, EB and CLIP170. The EEY/F motif is the target of CAP-Gly domains.
Dynein–dynactin complex: dynein is a large minus-end-directed multi-subunit microtubule motor that is involved in numerous cellular processes. Dynactin is an accessory multi-subunit complex of dynein that is important for dynein activation and its interaction with cargo.
End-binding-homology (EBH) domain: ~50 amino acid domain found at the C-terminus of EB proteins. It comprises a pair of helix–loop–helix segments forming an antiparallel four-helix bundle. A deep hydrophobic cavity on the surface of the EBH domain forms an interaction site for binding partners.
Golgi apparatus: an organelle that forms part of the endomembrane system in eukaryotic cells. Its primary function is to process and package the macromolecules that are synthesized by the cell. The Golgi apparatus is particularly important in the processing of proteins destined for secretion.
Kinesin: a microtubule-based molecular motor, most often directed towards the plus end of microtubules.
Kinetochores: specialized regions on chromosomes that are connected to microtubules and motor proteins during cell division in eukaryotes. Kinetochores function in the separation of chromosome pairs.
Leucine-rich repeat: a protein structural motif that forms an α/β horseshoe fold. It is composed of repeating 20–30 amino acid stretches that are unusually rich in the hydrophobic amino acid leucine.
Microtubule minus ends: the slow-growing microtubule ends *in vitro*. Minus-ends usually do not grow *in vivo*; they serve as sites of depolymerization or are stabilized and are often associated with the centrosome in the cell interior.
Microtubule plus ends: the microtubule ends that grow fast *in vitro*. *In vivo*, plus ends are the dynamic ends of microtubules that alternate between periods of growth and shrinkage and that are often directed towards the cell surface.
Palmitoylation: post-translational covalent attachment of fatty acids, such as palmitic acid, to cysteine residues in proteins.
Rescues: the transitions from microtubule shortening to growth.
Ubiquitin: a highly-conserved regulatory protein that is ubiquitously expressed in eukaryotes. Ubiquitylation refers to the post-translational modification of a protein by the covalent attachment of one or more ubiquitin monomers. The most prominent function of ubiquitin is targeting proteins for proteasomal degradation.
Zinc knuckles: protein domains in which specifically positioned cysteine and histidine residues coordinate the binding of a zinc ion, thereby generating a structural conformation (the 'knuckle') that is capable of protein–protein and protein–DNA interactions.

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Protein family	Molecular organization	Main functions	CAP-Gly-domain binding partners
CLIP170 Tip1p (*Sp*), Bik1p (*Sc*), D-CLIP-190 (*Dm*)		MT rescue and stabilization; targeting of dynein to MT ends; MT interaction with the cell cortex and kinetochores	MT, EB, p150^{glued}
CLIP115		MT rescue	MT, EB
CLIPR-59		Trafficking between endosomes and trans-Golgi network	Tubulin, MT?
CLIP4		n.k.	n.k.
Dynactin1 (p150^{glued}) Ssm4p (*Sp*), Nip100p (*Sc*), NudM (*An*)		Binding dynein to cargo; regulation of dynein processivity	MT, EB, CLIP170
TBCB Alf1p (*Sc*), Alp11p (*Sp*), F53F (*Ce*)		Biogenesis of α- and β-tubulin and regulation of its turnover	α-tubulin
TBCE Pac2p (*Sc*)		Biogenesis of α- and β-tubulin and regulation of its turnover	α-tubulin
CYLD dCYLD (*Dm*)		Deubiquitylation of Lys63-linked polyubiquitin chains; inhibition of NF-κB and JNK pathways	NEMO, MT
KIF13B (GAKIN) Khc-73 (*Dm*)		Transport of PIP_3-containing vesicles and tumour suppressor Dlg	n.k.
CAP350		MT anchoring at the centrosome	n.k.

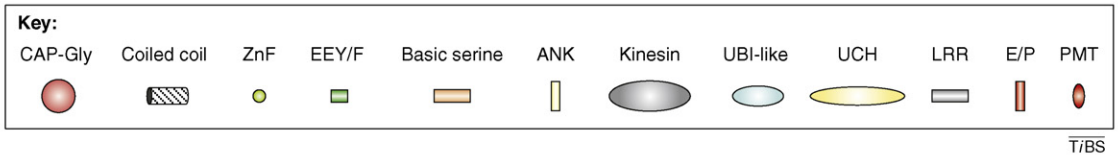

Figure 1. CAP-Gly-protein families and their properties. Unless otherwise indicated, the names of mammalian CAP-Gly proteins are given in bold. The list of orthologues is not comprehensive and includes only species-specific names of proteins for which some functional information is available. Abbreviations: An, *Aspergillus nidulans*; ANK, ankyrin repeats; Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; E/P, glutamate-proline rich; PMT, palmytoylation membrane-targeting motif; LRR, leucine-rich repeat; MT, microtubule; n.k., not known; Sc, *Saccharomyces cerevisiae;* Sp, *Schizosaccharomyces pombe;* UBI, ubiquitin; UCH, ubiquitin carboxyl-terminal hydrolase catalytic domain; ZnF, zinc finger domain. The question mark indicates a potential interaction of which the functional relevance is not clear.

and dynamic properties of CAP-Gly domains and compare their protein-tail binding mode with that of PDZ domains (named after its discovery in the postsynaptic density protein of 95 kDa [PSD-95], the *Drosophila melanogaster* tumour suppressor Dlg1 and the tight junction protein Zo-1). Finally, we discuss CAP-Gly domain defects that are involved in hereditary human diseases.

Functions of CAP-Gly proteins

The most evolutionarily conserved property of CAP-Gly proteins is their capacity to interact with tubulin monomers, tubulin dimers and/or microtubules (Figures 1 and 2). The microtubule-binding activity of CAP-Gly domains was described for the first time for CLIP170, the first representative of a protein family encompassing four members. CLIP170 was originally proposed to link endosomes to microtubules [7] and later was identified as the first +TIP [8]. Subsequent studies demonstrated that the CAP-Gly domains of mammalian CLIP170, of its homologue CLIP115 and of its yeast orthologues Bik1p (*Saccharomyces cerevisiae*) and Tip1p (*Schizosaccharomyces pombe*) are responsible for the localization of these proteins to microtubule plus ends through interactions with tubulin and end-binding (EB) protein 1 (for reviews, see Refs [4,5]). CLIP170, CLIP115 and their orthologues stabilize microtubules by preventing catastrophes (switches from microtubule growth to shrinkage) or by stimulating rescues (opposite transitions) [9–13]. In addition, CLIP170 participates in the

Box 1. Microtubule plus-end tracking proteins (+TIPs)

Microtubules are hollow polymeric tubes built from dimers of α- and β-tubulin: they are formed and disassembled by the addition and removal of tubulin dimers from their ends. Microtubule filaments are intrinsically polarized and contain two structurally distinct ends: the plus end (the rapidly growing end *in vitro* and the only growing end *in vivo*) and the minus end (the slowly growing end *in vitro*; *in vivo*, it can be stabilized or serve as a depolymerization site).

Microtubules are often highly dynamic and can rapidly switch between periods of growth and shortening. When microtubules grow, their plus ends accumulate a specific set of proteins known as microtubule plus-end tracking proteins, or +TIPs [2,73]. The most evolutionarily conserved family of +TIPs are end-binding proteins (EB1 and its homologues). EBs contain an N-terminal microtubule-binding domain and a C-terminal domain that mediates dimerization and protein–protein interactions. Similar to α-tubulin, EBs harbor a C-terminal EEY/F sequence motif. EBs directly bind to the majority of other known +TIPs and they are required for the accumulation of +TIPs at microtubule ends [2]. Other well-studied +TIPs include CLIP170 and the dynactin large subunit p150^{glued}, which both contain CAP-Gly domains that bind to EB protein C-terminal domains via the EEY/F motifs.

+TIPs accumulate at the growing microtubule ends in a comet-like pattern, showing maximal concentration at the freshly polymerized tip (Figure I). The mechanism underlying this localization is not yet fully elucidated. For some +TIPs, including the yeast CLIP170 orthologues, microtubule plus-end accumulation depends on kinesin-driven transport; however, this mechanism does not apply to mammalian CLIPs [2]. Measurements of the dynamics of EBs and CLIPs in mammalian cells showed that they exchange very rapidly at the growing microtubule tips, indicating that these proteins recognize a specific microtubule end structure that is formed during polymerization [58].

Recently, the plus-end tracking system from fission yeast, including the CLIP and EB orthologues Tip1p (Tip elongation protein 1) and Mal3p (microtubule integrity protein) and a plus-end-directed kinesin, Tea2p (tip elongation aberrant protein 2), was reconstituted *in vitro* from purified components [9]. These experiments showed that Mal3p, alone, can recognize growing microtubule ends. Mal3p rapidly exchanges at the growing ends, probably by preferentially binding to a specific microtubule end structure. By contrast, Tip1p requires both Tea2p and Mal3p for efficient plus-end accumulation. These experiments, together with structural and cell biological information opened the way for a deeper understanding of the plus-end tracking mechanism.

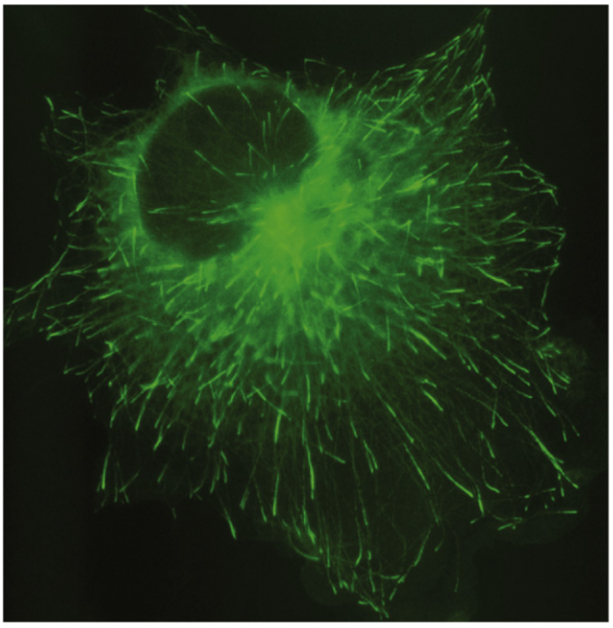

Figure I. CLIP170 at microtubule plus ends. The image illustrates the decoration of microtubule plus ends by GFP-CLIP170 in a COS-7 (African green monkey kidney) cell. COS-7 cells were transiently transfected with a GFP-CLIP170-expressing construct, fixed and imaged using a wide-field fluorescence microscope.

plus-end recruitment of the microtubule minus-end-directed motor cytoplasmic dynein. In vertebrates, the link between CLIP170 and dynein is most likely to depend on dynactin, a megadalton multi-subunit complex that functions as an accessory factor for cytoplasmic dynein (for a review, see Ref. [6]): CLIP170 associates with microtubules and EB1 through its CAP-Gly motifs, the dynactin large subunit p150^{glued} binds to the C-terminal zinc knuckles of CLIP170 and is recruited to the plus ends, and dynein associates with dynactin [4,14] (Figures 1 and 2). In budding yeast, the Bik1p CAP-Gly domain is also involved in recruiting dynein to microtubule tips, but this function does not involve dynactin [15]. Together with dynein, CLIP170 is also present at the kinetochores of mitotic cells, where it might participate in microtubule capture [16]. Participation in the dynein pathway might be the most important role of CLIP170 on the evolutionary scale because plants, which lack cytoplasmic dynein, do not encode CLIP170 orthologues in their genomes [17,18].

Although CLIP170 was originally proposed to link endosomal membranes to microtubules, this function was not elaborated further in later studies. By contrast, another member of the CLIP family, CLIP-related protein of 59 kDa (CLIPR-59), might be able to directly affect microtubules in the vicinity of membranes: the C-terminus of CLIPR-59 contains a palmitoylation membrane-targeting motif and two CAP-Gly domains associate with microtubules and tubulin [19,20] (Figure 2). CLIPR-59 localizes to the trans-Golgi network and plasma membrane, is involved in endosome-to-trans-Golgi trafficking and might regulate microtubule dynamics near certain membrane domains, for example lipid rafts [19,20]. The fourth member of the CLIP family in vertebrates is the poorly characterized CLIP4. This molecule comprises three CAP-Gly domains and, similar to CLIPR-59, contains ankyrin-repeat-like domains of unknown function [21].

Another well-conserved CAP-Gly protein is the already mentioned large subunit of dynactin, p150^{glued}. The dynactin complex is required for targeting dynein to cargo and for dynein motor processivity, but the underlying mechanism for this activity remains unclear. Based on *in vitro* data, the p150^{glued} microtubule-binding CAP-Gly domain might act as a 'brake' that inhibits dynein motility. Although the adjacent basic and serine-rich sequence region, which interacts with microtubules, enhances dynein processivity [22], the entire microtubule-binding domain of p150^{glued} does not seem to be required for normal dynein motility in cells [23]. Similar to CLIP170, p150^{glued} uses its CAP-Gly domain to accumulate at microtubule plus ends and at mitotic kinetochores, where the molecule contributes to dynein function (for a review, see Ref. [24]; Figure 2). p150^{glued} is also present at the centrosome where its

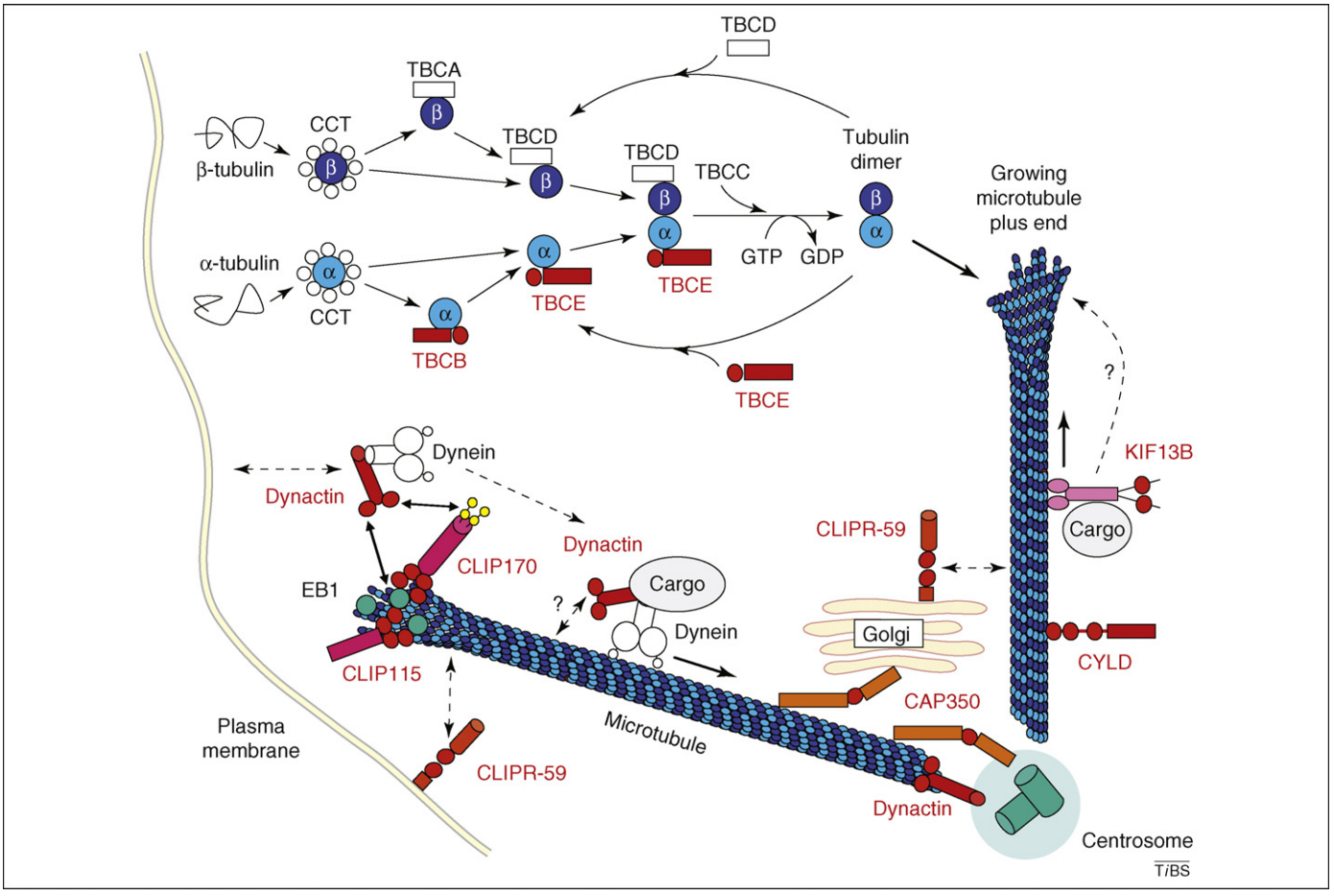

Figure 2. Cellular roles of CAP-Gly proteins. The most evolutionarily conserved property of CAP-Gly proteins is their ability to bind to tubulin monomers, tubulin dimers and/or microtubules. Various CAP-Gly proteins are shown in red; red circles denote CAP-Gly domains. The upper part of the figure schematically illustrates the tubulin heterodimer assembly pathway [27]. Tubulin-folding cofactors B and E (TBCB and TBCE) are involved in the folding and dimerization pathway of α-tubulin (light blue circle). Note that TBCE (in addition to TBCD) can also dissociate tubulin dimers into α- and β-tubulin (dark blue circle) monomers, leading to a reduction of the tubulin pool available for polymerization. CCT denotes cytosolic chaperonin, a ring-like protein complex involved in folding of actin and tubulin subunits. CLIP170 and CLIP115 target microtubule tips by recognizing the growing-end structure, which might contain tubulin sheets and an accumulation of EB proteins [2,4,58]. The large subunit of dynactin, $p150^{glued}$, can target microtubule plus ends through the interaction with the CLIP170 C-terminal domain or through interactions with the EBs and tubulin (discussed in Ref. [48]). Dynactin, in turn, can recruit cytoplasmic dynein (grey) to plus ends and, in this way, contribute to the loading of cargo for minus-end-directed transport or to interactions of microtubules with the plasma membrane. Note that CLIP115 lacks the zinc-knuckle-containing C-terminal domain (small yellow circles) that is present in CLIP170 and, thus, does not bind to the dynein–dynactin complex. Dynactin and CAP350 can anchor microtubules at the centrosomes (green) [26,37]. CAP350 might also stabilize microtubules at the Golgi [36]. CLIPR-59 might regulate the association between microtubules and the Golgi or the plasma membrane [20]. KIF13B transports cargo in the plus-end direction of the microtubule [38,39] and might also interact with microtubule tips [40]. CYLD interacts with microtubules and might regulate their dynamics [34]. Protein and organelle schemes are not drawn to scale. Proteins known or expected to be dimeric are shown as such; the remaining proteins are depicted as monomers. Established and less well-characterized interactions or functional transitions are indicated with solid and dashed arrows, respectively. The question mark indicates potential interactions of which the functional relevance is not clear.

CAP-Gly domain participates in anchoring microtubule minus ends [23,25,26].

The evolutionarily most conserved CAP-Gly proteins are the TBCB and TBCE. Through α-tubulin binding, both cofactors participate in tubulin dimer biogenesis and turnover; (for a review, see Ref. [27]; Figure 2). CAP-Gly domains in these proteins are required for their stable interactions with tubulin and with microtubules [27,28], but they do not seem to be essential for the tubulin-folding activity of the cofactors. The fission-yeast cofactor-B orthologue Alp11p (altered polarity protein 11) is required for viability; however, its absence can be rescued by a truncated protein missing the CAP-Gly domain [29]. Furthermore, the fission-yeast cofactor-E homologue Alp21p (altered polarity protein 21; also called suppressor of tsm one protein 1 [Sto1p]) lacks the CAP-Gly domain altogether [29]. Mammalian cofactor B is implicated in certain aspects of neuronal differentiation and its aberrant degradation causes defects in neural development [30]. It is likely that both cofactors regulate microtubule dynamics by controlling the abundance of tubulin dimers. In agreement with this idea, cofactor-E overexpression triggers microtubule network obliteration [31]. The roles, if any, of cofactors B and E in this process remain undefined.

CAP-Gly proteins also include the deubiquitylating tumour-suppressor protein CYLD. Through its C-terminal catalytical domain, CYLD hydrolyzes polyubiquitin chains on different proteins and thereby negatively affects signalling through nuclear factor κB (NF-κB), *c*-Jun N-terminal kinase (JNK) and possibly other pathways (for reviews, see Refs [3,32]). The third CYLD CAP-Gly domain is involved in targeting the enzyme to NF-κB essential modulator (NEMO) [33]. In addition, CYLD can associate with microtubules through its first CAP-Gly domain; this interaction might promote microtubule assembly, but no mechanism has been defined [34,35]. Further experiments are required

to determine whether microtubule binding is functionally linked to CYLD deubiquitylating activity.

The roles of the CAP350 and KIF13B CAP-Gly domains remain unclear. CAP350 is a large centrosomal protein [36,37]. Its single CAP-Gly domain does not bind to microtubules but instead probably mediates the interaction of CAP350 with the Golgi network [36]. CAP350 might be required for microtubule stabilization and/or anchoring at the Golgi apparatus and the centrosomes (Figure 2), although its exact function remains controversial [36,37]. KIF13B, a kinesin-3 family member, is a motor protein involved in transporting the tumour-suppressor protein discs large (Dlg) and phosphatidylinositol (3,4,5)-triphosphate-containing vesicles to the plus ends of microtubules [38,39]. The function of the KIF13B CAP-Gly domain is unknown, but, interestingly, its fly orthologue kinesin-73 (Khc-73), which functions together with Dlg in a cortical-polarity pathway, accumulates at the astral microtubule plus ends in neuroblasts [40].

Structures and interactions of CAP-Gly domains

CAP-Gly domains are characterized by highly conserved glycine and hydrophobic residues (Figure 3a; for consensus pattern, see Prosite [www.expasy.ch/prosite] entry PS00845). The pairwise sequence conservation across human CAP-Gly-domain homologues ranges between 20–85% and 35–95% sequence identity and similarity, respectively. The evolutionarily most conserved region encompasses the Gly-Lys-Asn-Asp-Gly (GKNDG) motif present in the second half of the sequence. Structures of an array of CAP-Gly domains originating from different proteins have been solved (Figure 3b) [41–47] (see also Protein Data Bank [www.rcsb.org/pdb] depositions by S. Yokoyama and coworkers). These structures revealed a novel globular-protein fold that approximates a highly twisted, five-stranded antiparallel β-sheet flanked by a small β-hairpin (Figures 3a,b and 4a). A unique cluster of conserved aromatic residues, which are packed against each other, forms a solvent exposed hydrophobic cavity bordered by the GKNDG motif (Figure 3a,c). Most of the characteristic glycine residues are involved in shaping the loop regions of the domain. Superposition of several CAP-Gly domains shows that their overall structures are similar (backbone root-mean-square-deviation ranges between 0.4–2.2 Å taking the $p150^{glued}$ CAP-Gly domain as the reference structure; Figure 3b).

A subset of CAP-Gly-mediated protein interactions have been analyzed in detail. These include the interactions formed between the $p150^{glued}$ CAP-Gly domain (p150CG) and the EB1 C-terminus (EB1c) [44], p150CG and the CLIP170 C-terminal Cys-Cys-His-Cys (CCHC)-type zinc-finger domain (ClipZnF) [45,47], the second CLIP170 CAP-Gly domain (CLIPCG2) and a C-terminal α-tubulin peptide (α3tub) [46], and the third CYLD CAP-Gly domain (CYLDCG3) and a NEMO C-terminal peptide (cNEMOp2) [42]. These studies revealed that isolated CAP-Gly domains display dissociation constants in the low micromolar range, typically between 2–20 μM. Similar affinities were also reported for the binding of the $p150^{glued}$, CLIP170 and CYLD CAP-Gly domains to tubulin and/or microtubules [34,48–50].

The atomic resolution structures of the p150CG–EB1c, p150CG–ClipZnF and CLIPCG2–α3tub complexes revealed that the highly conserved hydrophobic cavity of CAP-Gly domains encompassing the GKNDG motif serves as a binding site for C-terminal Glu-Glu-Tyr/Phe (EEY/F)-COO^- sequence motifs (referred hereafter as EEY/F; COO^- denotes the carboxylate group; Figure 3d). EEY/F motifs typically comprise five or more amino acids and are characteristic sequence signatures of α-tubulin and the +TIPs EB and CLIP170 [44]. The functional importance of CAP-Gly–EEY/F binding is clear from studies showing that mutations that target the GKNDG Lys-Asn dipeptide or residues within the EEY/F motif impair or abrogate CAP-Gly-domain interactions with their targets [43–47,51–56]. Together, these findings established that a prominent role of CAP-Gly domain is to bind C-terminal EEY/F protein tails.

The importance of a preserved GKNDG motif for binding C-terminal EEY/F protein tails is supported by the finding that the CAP350 CAP-Gly domain, in which the lysine and aspartate residues are replaced by asparagines (Figure 3a), does not interact with microtubules [36]. However, the first CYLD CAP-Gly domain also contains substitutions in the GKNDG motif (the lysine and asparagine residues are replaced by phenylalanine and threonine, respectively) even though it retains the capacity to interact with microtubules [34]. How these specific residue changes affect EEY/F binding or whether this particular CAP-Gly domain interacts with microtubules through another binding site remains untested.

Besides EEY/F, CAP-Gly domains have evolved to bind additional structural elements; the p150CG β2-β3 loop, for example, interacts with the hydrophobic cavity of the EB homology (EBH) domain within the p150CG–EB1c complex (Figure 3e). Because the sequence of this loop is variable among CAP-Gly proteins (Figure 3a), it determines EBH-binding specificity [44]. By analogy, non-conserved residues of the p150CG β2-β3 and β6-β7 loops also contact the ClipZnF zinc-knuckle moiety in the p150CG–ClipZnF complex (Figure 3f) and, therefore, determine the specificity of CAP-Gly binding to CCHC-type zinc-finger motifs [45,47]. It should be noted that these additional contact sites on these particular CAP-Gly domains can partially overlap (Figure 3b,f), explaining the observation that the interactions of $p150^{glued}$ with EB1 and CLIP170 are mutually exclusive [25,48,57]. A binary interaction mode between a CAP-Gly domain and its binding partner is likely to be necessary to achieve diverse specificities and functionally relevant complex stabilities [44,47]. In this context, binding of the short proline-rich cNEMOp2 peptide to the CYLDCG3 hydrophobic cavity was estimated to be of high micromolar to millimolar affinity [42]; whether an additional binding site contributes to the overall stability of the CYLD–NEMO complex remains untested.

CAP-Gly-domain interactions: regulation and functional implications

As discussed earlier, previously analyzed CAP-Gly-mediated protein interactions exhibit comparable dissociation constants in the low micromolar range. Because CAP-Gly domains are frequently involved in highly

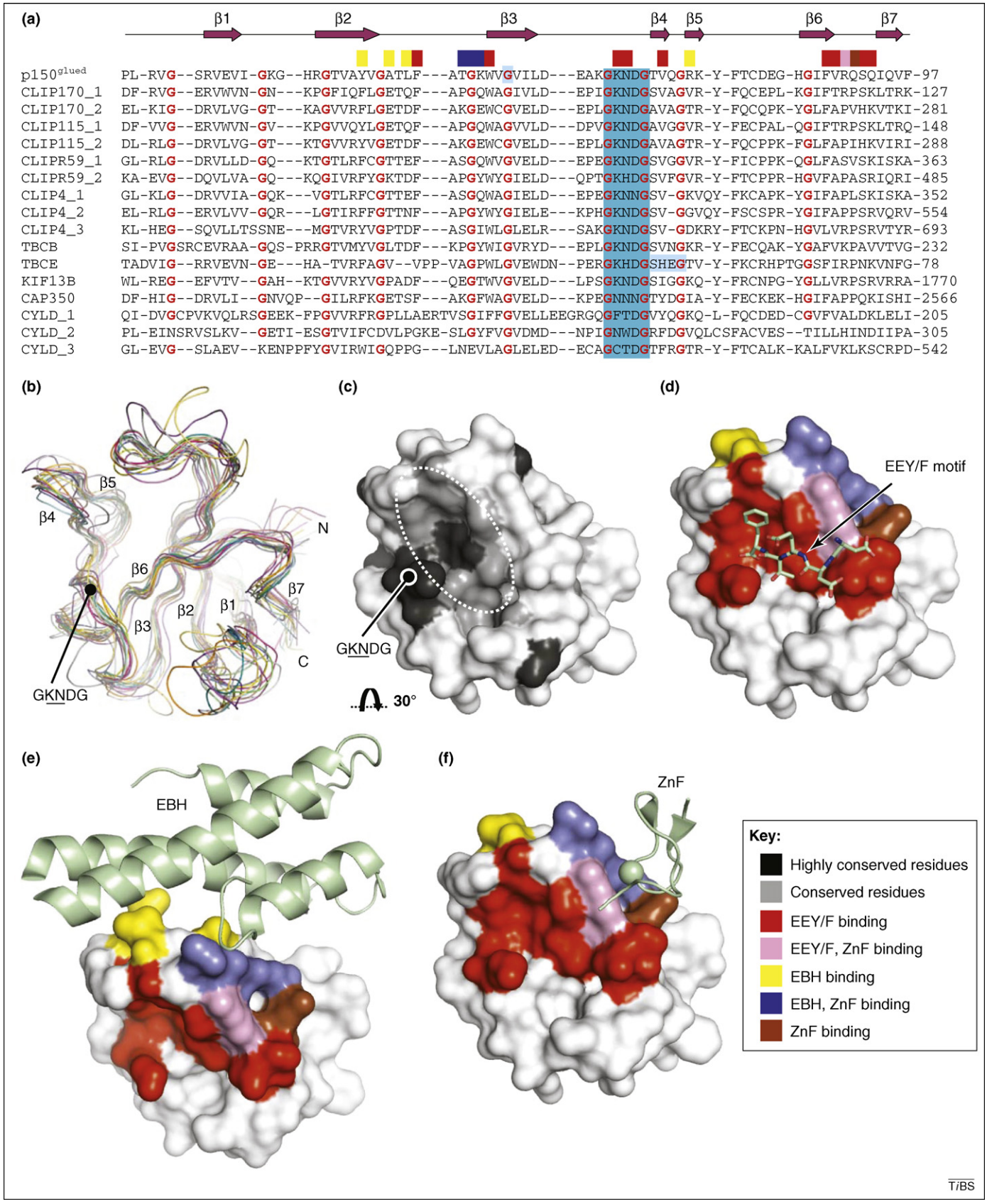

Figure 3. Sequence conservation, structure and binding modes of CAP-Gly domains. CAP-Gly domains are evolutionarily conserved protein modules that harbour specific sequence elements mediating protein–protein interactions. **(a)** Structure-based sequence alignment of human CAP-Gly-domain homologues. The glycine residues, characteristic for the CAP-Gly fold, are highlighted in red, bold font. Residues indicated on the top of the alignment in red and pink form contacts with the EEY/F motif, yellow and blue with the EB-like motif, and pink, blue and brown with the C-terminal zinc knuckles of CLIPs. Secondary structure elements are shown on top of the alignment. The highly conserved GKNDG motif is highlighted in turquoise. The Gly59Ser substitution in p150^{glued} found in a motor-neuron disease and the four-residue deletion in tubulin-folding cofactor E (TBCE) found in hypoparathyroidism-retardation-dysmorphism (HRD) syndrome are indicated in light blue. UniProtKB/Swiss-Prot (http://expasy.org/sprot) sequence accession numbers are as follows: p150^{glued}, Q14203; CLIP170, P30622; CLIP115, Q9UDT6; CLIPR59, Q96DZ5; CLIP4, Q8N3C7; TBCB,

dynamic and tightly regulated cellular processes, specific interactions of moderate affinities make sense: they are well suited to be continuously broken and reformed in response to rapid changes in their target structures, for example growing microtubule plus ends (Box 1). The interactions mediated by multiple CAP-Gly domains, either along the same or on different polypeptide chains, also seem ideal for the transient recruitment and clustering of different molecular functionalities. These properties are best illustrated by the +TIPs CLIP170, CLIP115 and p150^{glued}. These proteins use their CAP-Gly domains to transiently accumulate at growing microtubule plus ends by binding the C-terminal EEY/F motifs within α-tubulin, EB proteins and CLIP170 (for a review, see Ref. [2]). Binding to α-tubulin, EBs and CLIP170 tails might occur simultaneously because each p150^{glued} or CLIP molecule forms coiled-coil-domain-mediated dimers that contain two and four CAP-Gly domains, respectively (Figure 2). These interactions must be short-lived because both CLIPs and EB proteins exchange rapidly at microtubule tips [9,58]. These considerations indicate that low-affinity binding sites in different combinations control the dynamics and remodelling of CAP-Gly-mediated +TIP protein networks.

Some CAP-Gly domains, including those in p150^{glued}, CLIPs and CAP350, are surrounded by flexible basic and serine-rich sequence regions (Figure 1). These domains contribute to microtubule binding of CAP-Gly proteins, as reported for p150^{glued} [22] (see earlier) and CLIP115 [59], or to Golgi and centrosomal localization, as is the case for CAP350 [36]. Owing to the abundance of serine residues, these regions might function as phosphorylation-dependent regulatory elements.

The exquisite sensitivity of CAP-Gly domains for the presence of C-terminal aromatic residues can be exploited by cells to regulate the binding properties of CAP-Gly proteins. For example, the terminal tyrosine of α-tubulin can be removed enzymatically when tubulin dimers are incorporated into microtubules [2,60]. This well-known but until recently poorly understood post-translational process results in the exposure of the penultimate glutamate residue within the EEY/F motif and in the generation of detyrosinated (Glu) microtubules. After Glu microtubule disassembly, tyrosine can be re-added to tubulin dimers. Interestingly, CAP-Gly proteins, including CLIP170, CLIP115 and p150^{glued}, do not efficiently bind detyrosinated microtubules [56], in agreement with structural information demonstrating that the highly conserved hydrophobic cavity encompassing the CAP-Gly-domain GKNDG motif forms a specific binding site for the C-terminal aromatic residue within EEY/F motifs (Figure 3d; see also earlier). These findings indicate that an important potential role of the tubulin-detyrosination-tyrosination cycle is to regulate CAP-Gly-protein–microtubule interactions. As discussed earlier, EEY/F sequence motifs are also a characteristic feature of EB proteins and CLIP170; however, the post-translational removal of the aromatic C-terminal residue in these proteins has not been reported. It also remains untested whether other modifications, including polyglutamylation, that occur in the vicinity of EEY/F at the α-tubulin C-terminus [60] affect CAP-Gly-domain binding.

Box 2. C-terminal sequence-recognition domains

Protein C termini are unique protein-sequence signatures that, through their interactions with other proteins, are involved in a variety of biological processes, including signalling, protein targeting, subcellular anchoring and the formation of static and dynamic protein networks. These C-terminal sequences can serve directly as a binding site or become accessible after specific post-translational cleavage or modification events (for a review, see Ref. [74]). Various evolutionarily conserved protein domains have been identified that bind protein C-termini, including the PDZ, TPR (tetratrico peptide repeat), 14–3-3 (protein domain named after the characteristic migration pattern on electrophoretic gels), WW (protein domain named after two Trp residues) and, most recently, CAP-Gly domains. Proteins containing one or more such interaction modules with either identical or different binding specificities typically constitute molecular scaffolds capable of organizing protein networks and macromolecular complexes that assist signalling efficiency or provide binding sites for the localization of proteins to specific cellular structures. However, there are also examples known where C-termini binding domains directly regulate the activity of the target proteins. One such example is the interaction between the chloride-channel cystic-fibrosis transmembrane-conductance regulator (CFTR) and CFTR-associated protein of 70 kDa (CAP70). The multivalent binding of CAP70 PDZ domains to CFTR triggers transient CFTR oligomerization. This assembly process induces the formation of a more favourable conductance state of CFTR leading to enhanced chloride-channel activity [75].

Comparison of CAP-Gly and PDZ binding to C-terminal protein tails

In addition to CAP-Gly, other protein-domain families can serve as modules that bind to protein C termini (Box 2). A prominent and extensively studied example is the PDZ domain (for a review, see Ref. [61]). In contrast to CAP-Gly domains, which are found in a limited number of proteins, PDZ domains are among the most common protein modules. Whereas CAP-Gly domains seem to prefer acidic-aromatic sequence motifs, for example EEY/F, most PDZ domains bind peptide ligands that contain polar and predominantly small hydrophobic side chains (for example, the canonical Ser/Thr-Xaa-Val motif of class I PDZ). PDZ domains occasionally bind to internal peptide sequences at their carboxylate binding site that assume a non-terminal hairpin-turn conformation (for a review, see Ref. [61]); such

Q99426; TBCE, Q15813; KIF13B, Q9NQT8; CAP350, Q5VT06; CYLD, Q9NQC7. **(b)** Superposition of CAP-Gly-domain structures in ribbon representation. Protein Data Bank (PDB) entries are as follows: p150^{glued}, 2HKN (green); CLIP170_CG1, 2E3I (cyan); CLIP170_CG2, 2E3H (violet); CLIP115_CG1, 2CP2 (yellow); CLIP115_CG2, 2CP3 (pink); CLIPR59_CG1, 2CPO (light grey); CLIPR59_CG2, 1WHH (mid-grey); TBCB, 1WHG (orange); KIF13B, 2COW (pale green); CAP350, 2COZ (pink); CYLD_CG1, 1WHL (pale yellow); CYLD_CG2, 1WHM (purple); CYLD_CG3, 1IXD (dark grey). **(c)** Mapping of conserved surface residues [derived from panels (a) and (b)] onto the CAP-Gly fold. Black and grey represent highly conserved and conserved residues, respectively. The conserved hydrophobic cavity is indicated by the dashed oval. **(d,e,f)** Complexes formed between the p150^{glued} CAP-Gly domain (p150CG) and the CLIP170 C-terminal EEY/F sequence motif (d) (PDB entries 2PZO and 2HQH), p150CG and the end-binding-homology (EBH) domain (e) (PDB entry 2HKQ), and p150CG and the CLIP170 C-terminal zinc finger domain (ZnF) (f) (PDB entries 2PZO and 2HQH). The CAP-Gly domains are shown as surface representations. The binding partners are coloured in pale green and shown in sticks (EEY/F motif), cartoon (EBH and ZnF) and sphere (Zn atom) representations. The location of the GKNDG motif Lys-Asn dipeptide is indicated in panels (b) and (c). The CAP-Gly domains shown in panels (b), (c), (d) and (f) are identically oriented. The residue colour code used in all panels is depicted on the bottom right.

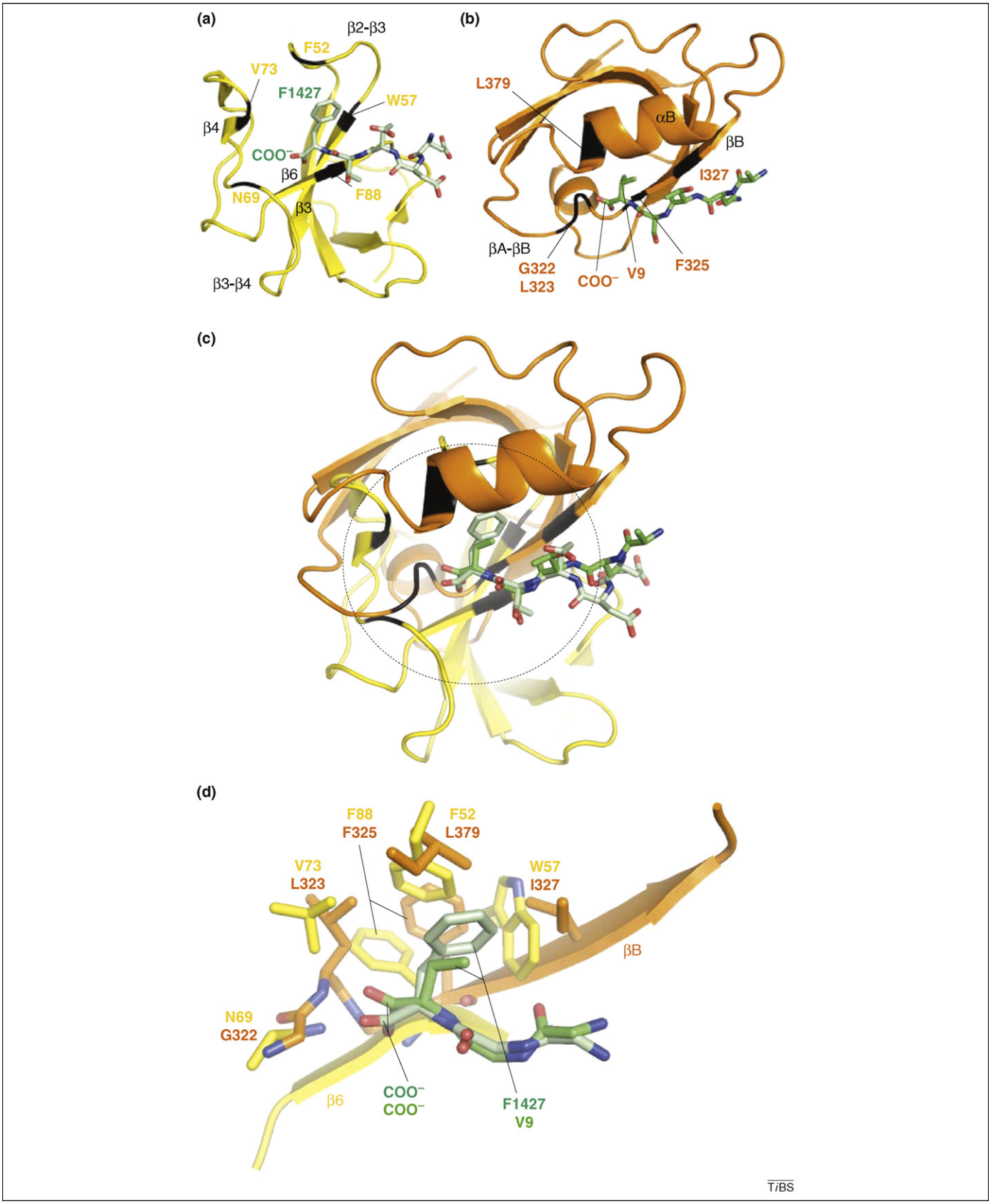

Figure 4. Comparison of CAP-Gly and PDZ domains. Although the architecture of CAP-Gly and PDZ are distinct, both domains are designed for binding hydrophobic residues with free carboxylate groups at the ends of protein tails. **(a)** The $p150^{glued}$ CAP-Gly domain (yellow ribbon) bound to the CLIP170 C-terminal DDETF-COO^- peptide (pale green sticks; PDB entries 2PZO and 2HQH). **(b)** The PSD-95 PDZ-3 domain (orange ribbon) bound to the CRIPT KQTSV-COO^- peptide (green sticks; PDB entry 1BE9). The positions of CAP-Gly and PDZ residues involved in the binding of the terminal phenylalanine and valine residues (including the carboxylate groups, COO^-) of the CLIP170 and CRIPT peptides, respectively, are highlighted in black. Secondary structure elements shaping the peptide ligand-binding site are indicated. The CAP-Gly and PDZ complex structures have been aligned by superimposing the backbone atoms of the three C-terminal residues of the CLIP170 and CRIPT peptide ligands. **(c)** Superimposition of the CAP-Gly and PDZ complex structures shown in (a) and (b). The carboxylate binding sites of the CAP-Gly and PDZ-domain

a binding mode at the EEY/F-binding site has not been described for CAP-Gly domains.

PDZ domains typically consist of a six-stranded antiparallel β-sandwich flanked by two helices. Although their overall folds are distinct from CAP-Gly domains, both display similarities in their manner of binding to C-terminal protein tails. In the following example, the CAP-Gly p150CG–ClipZn2 complex structure [45,47] (Figure 4a) and the complex formed between the third postsynaptic density protein of 95 kDa (PSD-95) PDZ domain (P95PDZ) and the C terminus of the cysteine-rich interactor of PDZ three (CRIPT) [62] (Figure 4b) are used as canonical representatives for the comparison. The topologies of the peptide ligand-binding sites are distinct: in p150CG it encompasses the β2-β3, β3-β4 and β6-β7 loops and strands β3, β4 and β6, whereas for P95PDZ it includes the βA-βB loop, the βB strand and helix αB (Figure 4a,b). However, in both complex structures the peptide ligands, Asp-Asp-Glu-Thr-Phe (DDETF) for ClipZn2 and Lys-Gln-Thr-Ser-Val (KQTSV) for CRIPT, assume an extended conformation and pack in an antiparallel manner against the β6 and βB strands of p150CG and P95PDZ, respectively. The main-chain moieties of the highly conserved Phe88 (p150CG) and Phe325 (P95PDZ) form hydrogen bonds with the carboxylate groups and main-chain moieties of the terminal Phe1427 (for ClipZn2) and Val9 (for CRIPT) residues (denoted as the 0-position residues; Figure 4c,d), respectively. An additional hydrogen bond to the ClipZn2 and CRIPT carboxylate groups is established with the Asn69 side-chain NH_2 group within p150CG and with the Leu323 main-chain NH group within P95PDZ, respectively. Finally, the side chains of the Phe1427 and Val9 residues at the 0 position of ClipZn2 and CRIPT, respectively, pack against a cluster of structurally conserved hydrophobic side chains that shape a similar cavity (Figure 4d).

Together, these considerations indicate that the architectures of CAP-Gly and PDZ domains, although distinct, are primarily designed for binding hydrophobic residues with free carboxylate groups at the ends of protein tails. Whereas most CAP-Gly domains seem to be selective for aromatic residues at the 0 position in peptide ligands [43–47], PDZ domains have evolved into several classes that prefer different terminal hydrophobic residues [61]. Remarkably, the CAP-Gly- and PDZ-domain carboxylate binding sites, which are well conserved among their homologues, are shaped by a structurally similar set of polar and hydrophobic atoms emanating from both common and distinct main-chain and side-chain polypeptide-chain elements (Figure 4d). The preference of many CAP-Gly domains for acidic residues upstream of the 0 position can be explained by the enrichment of basic residues, including the highly conserved Lys68 of the GKNDG motif, within or adjacent to the carboxylate binding site [46].

CAP-Gly-domain defects implicated in human diseases

Given the importance of CAP-Gly domains in central cellular processes, it is not surprising that mutations within their genes are linked to genetic human diseases. A well-studied example is the substitution of the highly conserved Gly59 residue for serine in $p150^{glued}$ (Figure 3a), which is associated with an autosomal dominant, slowly progressing motor-neuron disease (distal spinal and bulbar muscular atrophy) [63]. This substitution causes the $p150^{glued}$ CAP-Gly domain to misfold, probably owing to a steric packing problem of the mutant serine side chain within the domain core. As a consequence, binding of dynactin to microtubules and to CLIP170 is severely impaired [48,63]. Additionally, this single amino acid substitution causes the formation of cytotoxic dynactin aggregates both in tissues from patients and in cultured cells [64,65]. Several mouse studies confirmed the deleterious effect of this mutation, which has both loss-of-function and dominant-negative properties; it is embryonically lethal when homozygously present in knock-in mice [66] and causes a motor-neuron disease in hemizygous knock-in animals and in transgenics [66–68]. The disease is associated with different abnormalities, such as vesicle trafficking, neuromuscular junction defects and lysosome proliferation [66–68].

A four-residue deletion in the TBCE CAP-Gly domain (Figure 3a) causes a devastating and fatal autosomal recessive disorder manifested by hypoparathyroidism, mental retardation and facial dysmorphism (HRD) [69]. This deletion, adjacent to the highly conserved GKNDG motif (Figure 3a), is expected to perturb EEY/F binding within the CAP-Gly domain (Figure 3d). α-tubulin levels are not affected in homozygously mutated cells; microtubule network organization, however, is negatively affected. Another allele of the TBCE-encoding gene associated with the same syndrome harbours a mutation that results in the production of an N-terminally truncated protein, which lacks the β1, and a part of the β2 strand of the CAP-Gly domain. Although associated with disease, this mutated protein retains partial function [70].

Human mutations that abrogate the function of the tumour suppressor CYLD result in familial cylindromatosis, an autosomal dominant genetic disease characterized by the development of benign skin and hair follicle tumours, for example cyllindroma and trichoepithelioma (for reviews, see Refs [3,32]). The majority of these mutations cause premature translation termination and abolish the deubiquitylation activity (which is located at the C terminus), indicating the crucial nature of this enzymatic activity in CYLD cellular function [3,32]. No disease-linked mutations in the CYLD CAP-Gly domains have been isolated; however, the third CAP-Gly domain, which binds NEMO, seems to be important for normal function. Mice engineered to express exclusively a CYLD form that lacks this CAP-Gly domain and the adjacent binding site for the tumour-necrosis-factor (TNF)-receptor-associated factor 2 (TRAF2) display dramatic defects in B lymphocyte homeostasis [71]. Furthermore, the third CYLD CAP-Gly domain is required for regulating JNK signaling in *D. melanogaster* [72]. Whether and how CYLD

complexes are indicated by the dashed circle. **(d)** A high-magnification view of the superimposed carboxylate binding sites of the CAP-Gly and PDZ domain complexes shown in (c). For simplicity, only the side chains of the C-terminal residues of the two peptide ligands are shown. Oxygen and nitrogen atoms of depicted residues in sticks representation are coloured in red and blue. Carbon atoms are coloured in yellow (CAP-Gly), orange (PDZ), pale green (DDETF-COO^-) or green (KQTSV-COO^-).

signalling and tumour-suppressor activities are linked to its microtubule association requires further investigation.

Concluding remarks

CAP-Gly proteins perform important roles in intracellular signalling and transport, chromosome segregation, cell migration and tumourigenesis. Recent structural and functional studies have revealed the mechanisms of CAP-Gly-domain interactions with the C-terminal tails of α-tubulin, EBs and CLIP170 and how alternative sites contribute to CAP-Gly-binding specificity. Additionally, these studies have begun to unravel the many biological and clinical implications of this specialized protein module. Future work should determine whether CAP-Gly domains bind to and regulate additional +TIPs or other proteins that contain either C-terminal acidic-aromatic or alternative motifs. It will also be important to define how the preference for tubulin monomers, tubulin dimers, growing microtubule ends or the mature microtubule lattice by certain CAP-Gly proteins, for example the tubulin-folding cofactors, CLIPs and $p150^{glued}$, is regulated and how these interactions affect microtubule structure and dynamics. In this context, assessing the role of sequence regions flanking CAP-Gly domains and the effect of C-terminal tubulin modifications besides tyrosination on the modulation of CAP-Gly-protein activity is of particular interest. CAP-Gly domains are further involved in the assembly of protein-interaction networks and it remains to be determined how they contribute to the architecture and dynamics of these assemblies. Studies of CAP-Gly proteins provide an intriguing example of how the investigation of structural and functional properties of a small protein module by an integrated approach can contribute to a deeper understanding of a variety of central cellular processes.

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