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CLIP170 autoinhibition mimics intermolecular interactions with p150^{Glued} or EB1

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CLIP170 and p150^{Glued} localize to the plus ends of growing microtubules. Using crystallography and NMR, we show that autoinhibitory interactions within CLIP170 use the same binding determinants as CLIP170's intermolecular interactions with p150^{Glued}. These interactions have both similar and distinct features when compared with the p150^{Glued}–EB1 complex. Our data thus demonstrate that regulation of microtubule dynamics by plus end–tracking proteins (+TIPs) occurs through direct competition between homologous binding interfaces.

+TIPs associate with the growing ends of microtubules (MTs)[1], where they control MT dynamics and provide a bridge to MT targets such as the cell cortex or kinetochores[2,3]. However, the molecular mechanisms of +TIP regulation are poorly understood.

CLIP170, the prototypical +TIP, contains tandem N-terminal MT-binding glycine-rich cytoskeleton-associated protein (CAP-Gly) domains[4], which we term ClipCG1 and ClipCG2. Together with flanking serine-rich regions, the CAP-Gly domains are necessary and sufficient for plus-end tracking *in vivo*[5]. The two ClipCG domains are followed by a coiled-coil region that mediates homodimerization[6] and a C-terminal cargo-binding domain containing two zinc-finger motifs (ClipZn1 and ClipZn2).

p150^{Glued}, the MT-binding subunit of dynactin, comprises a single CAP-Gly domain (p150n) and a serine-rich region that together mediate plus-end tracking[7]. In cultured cells, dynactin colocalizes with CLIP170 in a 'comet-tail' pattern at MT plus ends[8,9], a process that requires a direct interaction between p150n and ClipZn2 (ref. 10).

CLIP170 can adopt a 'closed' conformation in which the CAP-Gly domains and the zinc-finger motifs form intramolecular contacts that inhibit MT binding[11]. This suggests that the switch from intramolecular to intermolecular CAP-Gly domain–zinc-finger motif complexes is important in the regulation of microtubule dynamics.

To explore this at a structural level, we first determined the crystal structure of human p150n in complex with ClipZn2 (**Fig. 1a,** and **Supplementary Fig. 1**, **Supplementary Table 1** and **Supplementary Methods** online). p150n adopts the β/β fold observed previously in the p150n–EB1 crystal structure[12,13]. ClipZn2 has an irregular fold that orients four side chains (Cys1408, Cys1411, His1416 and Cys1421) to provide tetrahedral coordination of the zinc ion. As might be expected for such a short motif, the integrity of the Zn^{2+} site is required for binding p150n[10,11].

Complex formation buries ~1,020 Å^2 of exposed surface and involves two distinct regions. The first comprises the zinc-finger motif and the lower loops of p150n. Here, p150n Arg90 has a central role both in the structural stability of the p150n domain itself—where it forms a cation-π interaction with Trp57 and hydrogen bonds to the main chain carbonyl groups of Thr54 and Lys56 (**Fig. 1a**,**b**)—and at the interface with ClipZn2, where its side chain approaches within 5 Å of the zinc ion. The positive charge on Arg90 is shielded by, and appears to interact favorably with, the distal side of the Cys1411 Sγ, which is polarized by its interaction with Zn^{2+}. The second region involves the C-terminal tail from the zinc-finger motif, whose last residue, Phe1427, inserts into a hydrophobic groove on the side of p150n; an analogous interaction has been observed between p150n and EB1 (ref. 13). The highly conserved 67-GKNDG-71

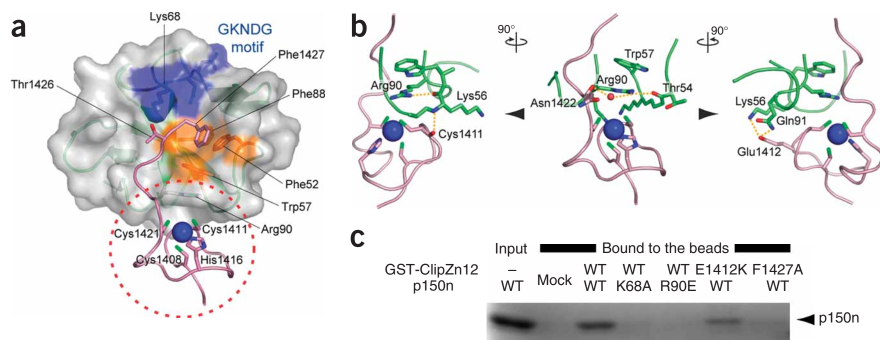

Figure 1 p150n-ClipZn2 interactions. (**a**) Surface representation of p150n with Cα traces of ClipZn2 (pink) and p150n (green). Key interacting side chains are shown as sticks (green atoms are sulfur). Blue, GKNDG motif; orange, conserved hydrophobic residues (Phe52, Trp57 and Phe88; **Supplementary Fig. 3** online). Red circled region is expanded in **b**. (**b**) Close-up views of interface between ClipZn2 and p150n. Middle panel has same orientation as in **a**. Residues discussed in the text are labeled. Orange sphere, water molecule mediating the Arg90-Asn1422 interaction. (**c**) Pull-down assays of wild-type and mutant ClipZn12 and p150n. p150n proteins were incubated with glutathione *S*-transferase (GST)-fused ClipZn12 proteins. Left lane contains wild-type p150n, for reference. Mock pull-down is shown as a control (see **Supplementary Table 2**).

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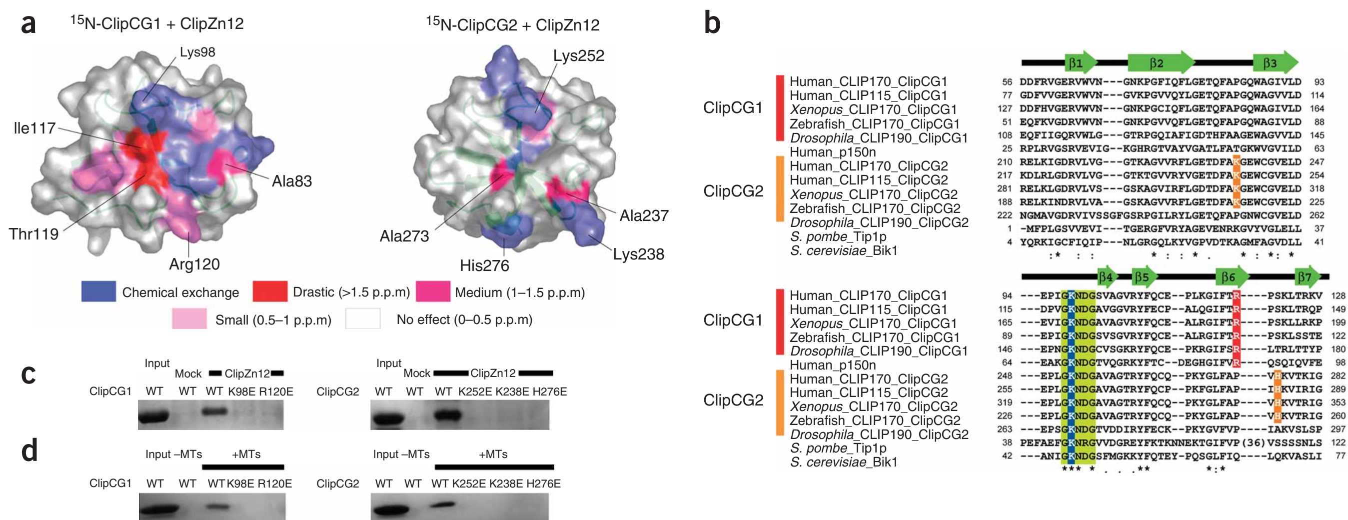

Figure 2 Recognition of ClipZn12 by ClipCG domains. (**a**) NMR chemical shift perturbations induced by binding of ClipZn12, mapped onto the surface of ClipCG1 (left; PDB 2CP7) and ClipCG2 (right; PDB 2CP6). The two panels show equivalent views. Purple residues are missing from the HSQC spectra owing to chemical exchanges. See **Supplementary Figure 2**. (**b**) Sequence alignments of ClipCG domains. ClipCG1 and ClipCG2 of human, *Xenopus laevis* and zebrafish CLIP170, human CLIP115 and *Drosophila* CLIP190 are shown. The single ClipCG domains of p150n, fission yeast Tip1p and budding yeast Bik1 are also shown. Green highlight, GKNDG motif; blue, its invariant lysine residue; red, arginine residues conserved in ClipCG1 (and p150n); orange, lysine and histidine conserved in ClipCG2. (**c**,**d**) *In vitro* assays of wild-type or mutant ClipCG1 and ClipCG2 binding to ClipZn12 (**c**; see **Supplementary Table 2**) or MTs (**d**). ClipCG1, ClipCG2 and mutants were detected by Coomassie staining after SDS-PAGE. Input lanes show wild-type ClipCG domains, for reference. All the mutations abrogate binding to ClipZn12 or MTs.

motif wraps around the top of the groove, with Lys68 forming a hydrogen bond with ClipZn2 Thr1426. Consistent with a crucial role for both regions in complex formation, point mutations at Lys68, Arg90 and Phe1427 abrogate binding (**Fig. 1c** and **Supplementary Table 2** online).

The zinc-finger motifs of CLIP170 form autoinhibitory interactions with its own CAP-Gly domains, which themselves regulate binding to MTs[11]. Using NMR, we mapped the ClipCG interaction surfaces by titrating individual ^{15}N-labeled CAP-Gly domains (ClipCG1 or ClipCG2) with the unlabeled zinc-finger motif pair (ClipZn12; **Fig. 2a** and **Supplementary Fig. 2** online). For ClipCG1, the perturbed surface coincides with analogous regions of p150n that bind ClipZn2. For example, Arg120, the analog of Arg90 in p150n, underwent a compound chemical shift change of 0.65 p.p.m., and most peaks in the GKNDG motif showed chemical exchange broadening. Although smaller perturbations were observed for ClipCG2, the resonances of the GKNDG motif were markedly broadened[14]. We further identified Lys238 and His276, conserved only in vertebrate ClipCG2 sequences, as a recognition site of ClipZn12 (**Fig. 2b**). These residues may give diversity to the target recognition of CAP-Gly domains in CLIP170.

We tested the binding models by charge-reversal mutagenesis (K98E and K252E from the two GKNDG motifs, R120E in ClipCG1, and K238E and H276E in ClipCG2). Each of the five mutations abrogated binding to ClipZn12 (**Fig. 2c** and **Supplementary Table 2**), consistent with our structural analysis. Furthermore, none of the mutants bound MTs (**Fig. 2d**), consistent with the conclusion that the binding sites for p150n, ClipZn12 and MTs overlap[12].

Together, our data show that the autoinhibitory conformation of CLIP170 and the interactions of CLIP170 with p150^{Glued} involve analogous and mutually exclusive intra- or intermolecular CAP-Gly domain–zinc-finger motif interactions[11,15]. Competition between these interactions provides a structural rationale for the dynamic localization and activation of these proteins, which are important for the organization of the MT network, MT-kinetochore attachment and MT minus end–directed motility.

Accession codes. Protein Data Bank: Coordinates have been deposited with accession code 2HQH.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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