Cryptic No Longer: Arrays of CLASP1 TOG Domains

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CLASP proteins play crucial roles in regulating microtubules. In this issue of *Structure*, Leano and colleagues show that an essential and previously cryptic domain of CLASP is a TOG domain with unusual features that might explain its unique functions.

Microtubules (MTs) are polymers made of tubulin heterodimers assembled into polarized protofilaments that associate laterally to form a hollow rigid tube about 25 nm in diameter. As a central component of the cytoskeleton, MTs play a prominent role in cell division when they form the mitotic spindle, the supramolecular structure responsible for segregating replicated chromosomes to daughter cells. A key feature of MTs is their ability to undergo cycles of growth and shrinkage and rapidly switch between these two states, a behavior termed dynamic instability (Mitchison and Kirschner, 1984). Although pure MTs exhibit dynamic instability, their transition frequencies and dynamic parameters are regulated in cells through the interaction of many MT-associated proteins (MAPs) (Desai and Mitchison, 1997).

An important class of MAPs are the cytoplasmic linker associated proteins (CLASPs). CLASP family proteins are associated with multiple cellular functions. They help organize subcellular structures by linking MTs to organelles and are required for proper spindle assembly during mitosis (Miller et al., 2009; Pereira et al., 2006). CLASPs are established regulators of MT dynamics with multiple MT-binding and -regulating domains, including conserved TOG domains that bind tubulin dimers and recruit them to MTs (Al-Bassam and Chang, 2011). The mechanisms by which these domains function together have not been established, and even the number of MT interacting domains remains poorly defined. However, in this issue of *Structure*, Leano et al. (2013) provide important new insight by determining the structure of a domain of human CLASP1, previously termed a cryptic TOG domain (or TOG-like domain 2) for its lack of clear TOG domain sequence homology. Furthermore, by systematically mutating residues of this domain in the *Drosophila* CLASP homolog, the authors demonstrate its importance for spindle assembly.

TOG domains consist of six HEAT repeats aligned into a wide, flat rectangular tertiary structure, with helices making up the wide face and inter-helix loops making up the thin face (Al-Bassam et al., 2007; Slep, 2009; Slep and Vale, 2007). Tubulin dimers bind along one thin edge of the rectangle, and most TOG domains occur in tandem arrays. A CLASP conundrum was that it contained only one clearly identifiable TOG domain, TOG1 (Slep, 2009) (Figure 1A). Although several studies had implicated additional regions of CLASP proteins in MT regulation (Al-Bassam et al., 2010; Patel et al., 2012), until now, it was unknown whether CLASP was simply an exception to the TOG array rule or if it contained multiple true TOG domains. Using X-ray crystallography, Leano et al. (2013) show that the human CLASP1 first cryptic TOG domain is indeed a bona fide TOG, but with a unique bent architecture. This domain has now lost its cryptic title and can be referred to as the CLASP1 TOG domain 2 (TOG2) (Figure 1A and B).

TOG2 possesses the canonical structure of six HEAT repeats, but unlike other TOG domains, it is separated into two triads, with the first three HEAT repeats separated by a hinge from the second triad (Figure 1B). The

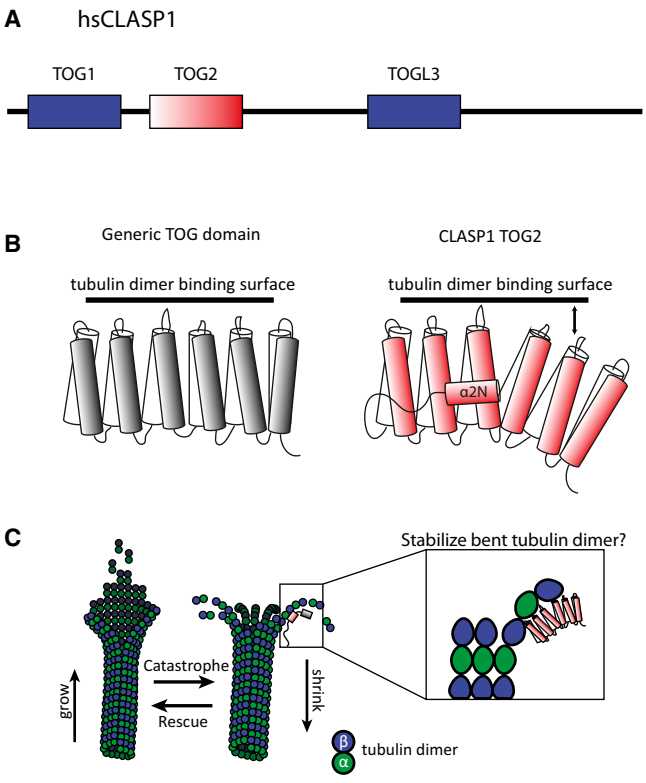

Figure 1. CLASP TOG Domain 2 Architecture May Confer Unique Activity to CLASPs Compared to Other TOG Domain-Containing Proteins
(A) TOG or cryptic TOG (TOG-like [TOGL]) domains found in human CLASP1.
(B) TOG2 adopts a bent architecture and is stabilized by an N-terminal helix (α2N).
(C) TOG2 may stabilize the depolymerizing MT by binding curved tubulin dimers in curling protofilaments.

hinge point breaks the surface into two shorter faces abutting each other at an angle, thereby generating a bent tubulin-binding surface. The authors go on to show that certain residues (W338, K423, and R462 in human CLASP1) known to bind tubulin in other TOG domains are required for colocalization of a minimal TOG array with MTs and for rescuing the collapse of the mitotic spindle in *Drosophila* S2 cells depleted of the endogenous protein. In vitro nucleation of MTs also requires these residues and suggests that, despite its unique bent architecture, TOG2 appears to use the same tubulin-binding surface as other TOG domains.

These interesting findings leave open the question of what the bent architecture represents. One possibility is that any TOG domain could adopt this conformation and that various conformations are involved in the binding and release of tubulin dimers thought to generate TOG activity. Another possibility proposed by the authors is that the bent conformation of the CLASP1 TOG2 domain is a structural feature that contributes to the unique functions of CLASP proteins. In the fission yeast *Schizosaccharomyces pombe*, CLASP family members can promote MT rescue and the switch from MT shrinkage to growth, and, in *Drosophila*, it can promote MT pausing, a transient state when the MT is neither growing nor shrinking. Tubulin dimers adopt a curved conformation as MT protofilaments peel away from the depolymerizing MT. The bent architecture of TOG2 might bind these highly curved tubulin dimers and stabilize protofilaments, thereby inhibiting MT shrinkage (Figure 1C), leading to a pause and potentially a rescue, if the MT end can regain a conformation compatible with polymerization.

In support of TOG2 having unique structure-function relationships compared to other TOG domains, an N-terminal helix (α2N) was observed to bind along the wide side of the rectangular tertiary structure: a feature not found in other TOG domain structures. The α2N helix appears to be pinned in place through the interaction of two phenylalanine residues, with pockets between helices in the first triad of TOG2. Mutation of either phenylalanine to glutamate or both residues to alanine led to severely destabilized TOG2, as judged by protein insolubility when expressed in bacteria and low expression levels in cultured cells, suggesting that TOG2 has unique structural features that diverge from other TOG domains.

Overall, this work provides important new insights into how TOG domains function. The identification of a second TOG domain in CLASP supports the model that TOG domains function in arrays and suggests that other cryptic TOG domains (or TOG-like domains) might be true TOG domains as well. Furthermore, the bent architecture of CLASP1 TOG2 highlights the need to determine whether TOG domains undergo large-scale conformational dynamics, a new avenue of study not implicated by previous structures. Finally, the authors have identified individual residues important for CLASP protein function in mitosis. These findings provide a strong foundation for further mechanistic studies of the role of CLASP proteins in spindle assembly and for their role in other MT-based cellular functions.

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