CLASP2 Has Two Distinct TOG Domains That Contribute Differently to Microtubule Dynamics

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Abstract

CLIP-associated proteins CLASPs are mammalian microtubule (MT) plus-end tracking proteins (+TIPs) that promote MT rescue *in vivo*. Their plus-end localization is dependent on other +TIPs, EB1 and CLIP-170, but in the leading edge of the cell, CLASPs display lattice-binding activity. MT association of CLASPs is suggested to be regulated by multiple TOG (*t*umor *o*verexpressed *g*ene) domains and by the serine-arginine (SR)-rich region, which contains binding sites for EB1. Here, we report the crystal structures of the two TOG domains of CLASP2. Both domains consist of six HEAT repeats, which are similar to the canonical paddle-like tubulin-binding TOG domains, but have arched conformations. The degrees and directions of curvature are different between the two TOG domains, implying that they have distinct roles in MT binding. Using biochemical, molecular modeling and cell biological analyses, we have investigated the interactions between the TOG domains and αβ-tubulin and found that each domain associates differently with αβ-tubulin. Our findings suggest that, by varying the degrees of domain curvature, the TOG domains may distinguish the structural conformation of the tubulin dimer, discriminate between different states of MT dynamic instability and thereby function differentially as stabilizers of MTs.

Introduction

Microtubules (MTs) are highly dynamic polymers of α- and β-tubulin heterodimers that play essential roles in mitosis, intracellular transport and cell polarity. The head-to-tail association of the tubulin dimer makes MTs polar structures, which is critical for the functions of MT arrays in cells. Every tubulin dimer is oriented with β-tubulin pointing toward the faster polymerizing plus-end of a MT and, following assembly, GTP hydrolysis occurs on the β-tubulin subunit. The MT plus-ends extend to the cell periphery, undergoing alternate phases of growth and shrinkage involving two transition states termed catastrophe and rescue, a process known as dynamic instability [1,2]. This intrinsic dynamic behavior enables the MT cytoskeleton to search the interior of the cell and capture targets, such as kinetochores, vesicles and the cellular cortex.

The balance between dynamically unstable and stable MTs is finely controlled by proteins that associate with either MTs or tubulin dimers. In particular, the proteins that accumulate at growing MT plus-ends are called MT plus-end tracking proteins (+TIPs) [3]. CLIP-associated proteins CLASPs, a family of +TIPs, localize at kinetochores, the spindle midzone, the Golgi apparatus and the cell cortex where they play multifunctional roles in mitosis and cell motility [4–7]. Because CLASP1α and CLASP2α possess a tubulin-binding module known as the TOG (*t*umor *o*verexpressed *g*ene) domain, first identified in the XMAP215/Dis family proteins, CLASPs are considered to be MT-stabilizing factors [7,8]. XMAP215, which contains five TOG domains at its N terminus, functions as a processive MT polymerase: XMAP215 binds tubulin dimers to facilitate their incorporation into MT plus-ends [9]. Crystallographic analyses showed that TOG domains form a "paddle" structure containing six pairs of

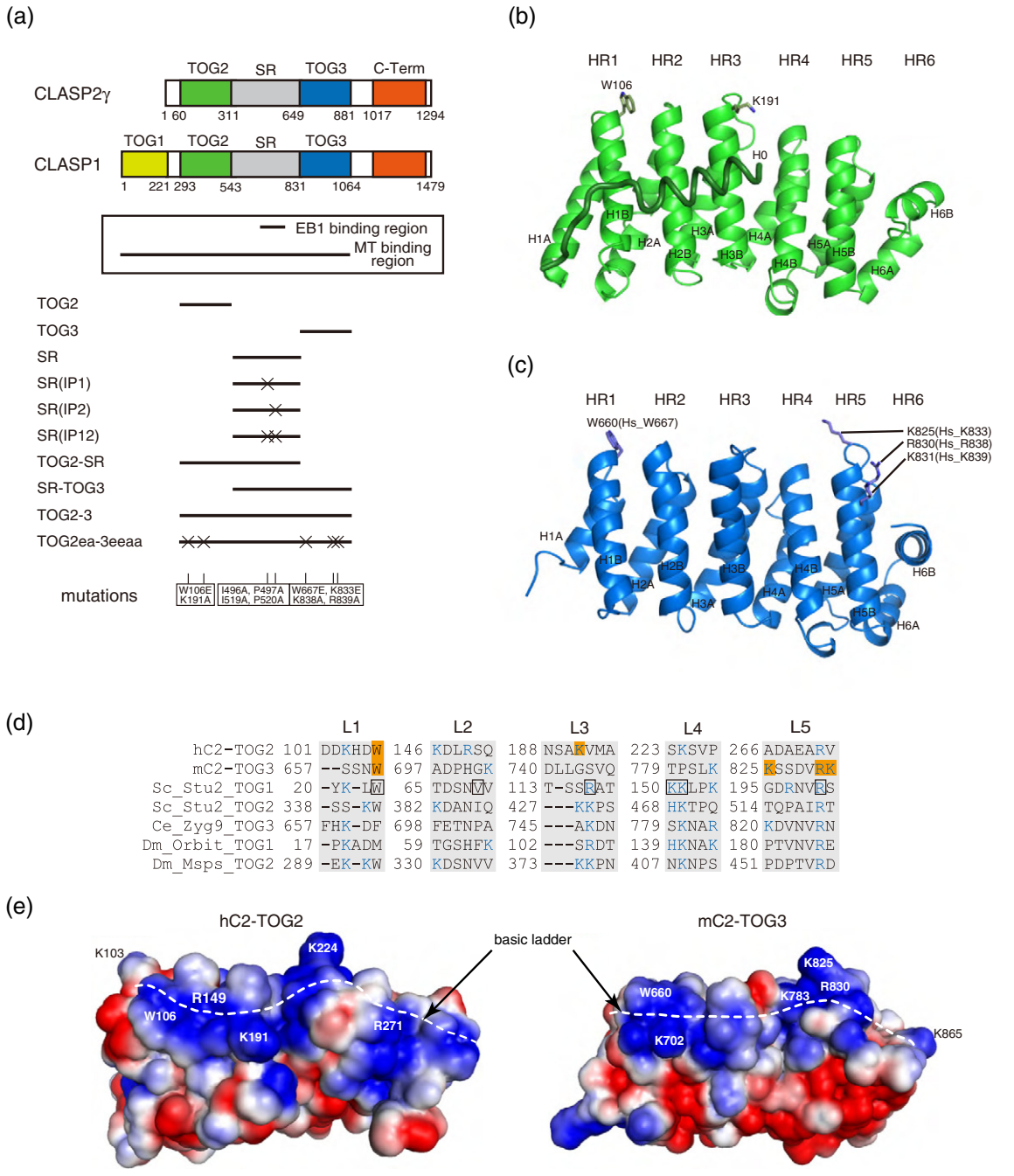

Fig. 1. Crystal structure of the TOG domains from CLASP2. (a) Domain organization of CLASP1 and CLASP2γ. EB1- and MT-binding regions are shown in the box. Schematics of the constructs of human CLASP2 tested in this *in vitro* study are shown below the box. Mutations used in this study are listed at the bottom. (b and c) Cartoon representation of hC2-TOG2 (b) and mC2-TOG3 (c). The H0 helix of hC2-TOG2 is shown as a coil. The N-terminal helices of HRs are labeled "A" and the C-terminal helices are labeled "B". Residues mutated in this study are shown in stick representation. In (c), mouse TOG3 residue numbers are labeled, and the corresponding numbers in human TOG3 are indicated in brackets. (d) Sequences of the tubulin-binding loops (L1-5) in TOG domains whose structures were determined previously: hC2-TOG2; mC2-TOG3; *Saccharomyces cerevisiae* (Sc) Stu2 TOG1 and TOG2; *Caenorhabditis elegans* (Ce) Zyg9 TOG3; *Drosophila melanogaster* (Dm) Orbit TOG1 and Msps TOG2. Residues mutated in this study are highlighted in orange. Basic residues forming a basic ladder are colored blue. The tubulin-binding residues seen in the Stu2-TOG1:tubulin complex are boxed in black. (e) Electrostatic potential of hC2-TOG2 (left) and mC2-TOG3 (right) countered from $-1.5\ kTe^{-1}$ (red) to $+1.5\ kTe^{-1}$ (blue), as calculated by APBS [52]. The orientation is the same as in (b) and (c). The H0 helix in hC2-TOG2 is not included. Residues discussed in the text are labeled. The basic ladders are indicated with white broken lines.