Structural basis for actin capping protein regulation by two different inhibitors

Actin-based cell motility is fundamental to various biological events such as neuronal development and immune response. Actin shows a highly dynamic behavior: monomeric actin reversibly assembles into a filamentous structure of actin filaments. Actin filaments have polarity and, in cells, their polymerization reaction occurs exclusively at the barbed end. When cells migrate, actin filaments concertedly elongate by pointing their barbed end toward the plasma membrane, which provides the driving force for the migration. Thus, the regulation of the barbed end is important for precisely controlled cell movement. The actin capping protein (CP) plays a key role in this process, since it binds tightly to the barbed end thereby stopping the elongation of the "capped" actin filaments [1]. CP is a heterodimeric protein composed of the α- and β-subunits and its crystal structure revealed that both subunits exhibit remarkably similar 3D architectures despite the lack of apparent similarity in their amino acid sequences (Fig. 1 and [2]). The α-tentacle is the major barbed end binding site in CP, located at the C-terminal region of the α-subunit (shown in red in Fig. 1). When CP caps the actin filament, it wedges between the two barbed end actin subunits with its α-tentacle region [3].

In motile cells, changes in the CP concentration lead to the formation of different actin cytoskeleton structures that cause alterations in cell shape and motility, indicating that the local concentration of CP and its affinity to the barbed end are key determinants for cellular actin assembly. Accordingly, cells exploit several proteins that can modulate the capping activity of CP. At present, V-1 and CARMIL are the only proteins that have been known to bind directly to CP and suppress its capping activity. V-1, also known as myotrophin, is a small ankyrin repeat protein implicated in a variety of cellular events such as muscle development and insulin secretion. CARMIL is a large, multi-domain protein and its down-regulation in amoeba and mammalian cells results in impaired cell motility. Previous studies demonstrated that the two inhibitory proteins affect the capping activity of CP in distinct manners. V-1 completely inhibits CP from interacting with the barbed end, whereas CARMIL acts on CP that has already capped the filament and facilitates its dissociation from the barbed end (uncapping activity). However, the molecular mechanisms underlying these differences are poorly understood. To address this important issue, we solved the crystal structures of CP in complex with V-1 or CARMIL. X-ray diffraction data were collected at **BL26B1** beamline and the structures of the crystals were determined at 1.7-2.2 Å resolutions.

Our results revealed that V-1 physically prevents CP from filament capping. V-1 directly interacts with the α-tentacle, the main actin binding site of CP, and therefore effectively sequesters CP from the barbed end (Fig. 2(a)). In contrast to V-1, CARMIL binds CP on an opposite surface to its actin binding site (Fig. 2(b)), indicating that CARMIL does not compete with the barbed end for the binding surface on CP. This finding is consistent with the ability of CARMIL to uncap the filament, since CARMIL can bind CP even when it caps the filament. However, this raises a fundamental question as to why the binding of CARMIL leads to an attenuated capping activity.

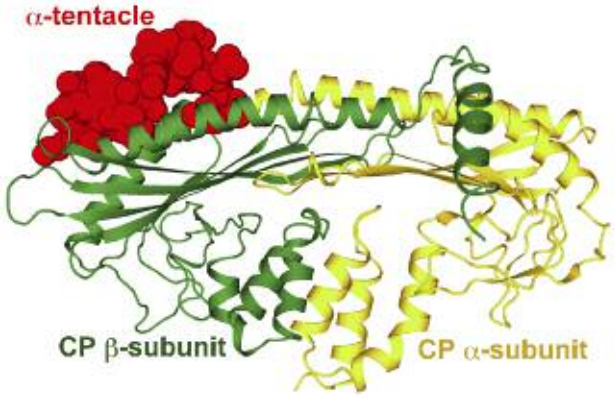

Fig. 1. Crystal structure of CP. The α- and β-subunits are shown in yellow and green, respectively. The main actin binding site, i.e., the α-tentacle, located at the C-terminal end of the α-subunit, is shown in red.

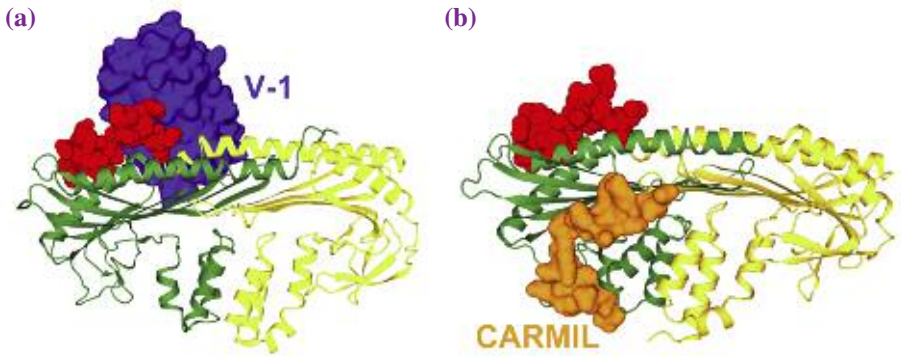

Fig. 2. Crystal structures of the CP/V-1 **(a)** and CP/CARMIL **(b)** complex structures. V-1 and CARMIL are shown in blue and orange, respectively. V-1 directly binds to the α-tentacle, demonstrating that V-1 physically prevents CP from barbed end capping. In contrast, CARMIL acts as an allosteric inhibitor for CP, since it does not physically obstruct actin binding.

Another important finding from our study provides a key to answer this question. A detailed comparison of several CP structures revealed that CP has two stable domains, CP-L and CP-S, which are continuously twisting relative to each other (Figs. 3(a) and 3(b)). This is a completely unexpected result, since CP had been considered as a rigid heterodimeric molecule. CARMIL simultaneously interacts with the two domains (Fig. 3(c)). We therefore concluded that CARMIL attenuates the binding of CP to actin filaments by suppressing the twisting motion required for tight barbed end capping.

Our results suggest that CP is not a constitutively active inhibitor of actin filament elongation; rather, its capping activity is fine-tuned for the highly orchestrated assembly of the cellular actin machinery and the conformational flexibility of CP provides the structural basis for the regulation.

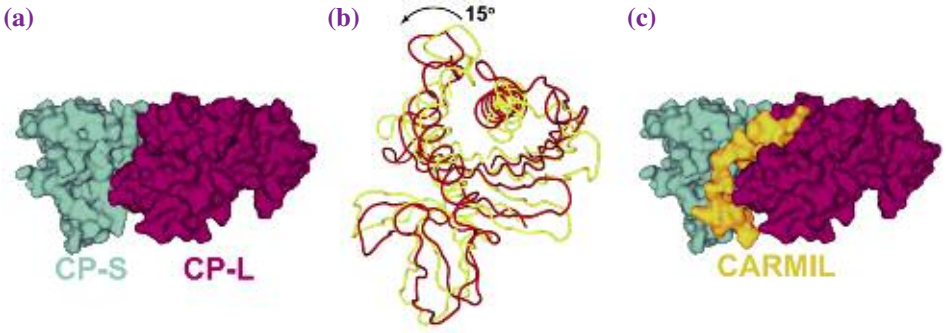

Fig. 3. Conformational flexibility of CP. **(a)** Two CP domains, CP-L and CP-S, are shown in purple and cyan, respectively. **(b)** Two different CP structures are superposed over the CP-L domain. CP-S domain structures, viewed from the left-hand side in (a), are shown in different colors and CP-L domain was removed for clarity. The CP-S domain rotates relative to the CP-L domain by about 15 degrees. **(c)** CARMIL (orange) binds to CP across the two domains, thereby restricting the twisting movement between them.

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