

## Actin Filament Capping Protein (CapZ): The Story After Crystal Structure Elucidation

Capping protein (CP), or CapZ in muscle cells, binds the fast growing B-end of an actin filament, thereby blocking the addition and removal of monomeric actin molecules at the B-end. In ordinary cells, various actin-binding proteins accelerate polymerization at B-ends and depolymerization at P-ends (Fig. 1), specifying the location and translocation of cellular organelles. This molecular movement, named the actin dynamics, requires CP, which reduces the number of uncapped B-ends and increases growth rate.

We started the protein crystallographic study of this protein, because we wanted to exploit the possibility of preparing actin minifilaments. Since CapZ is a naturally occurring protein that inhibits the elongation of actin filaments, knowledge of the capping mechanism could enable us to control filament length. A good start was made possible by the work by Obinata and coworkers, who established a good *E. coli* expression system for this protein [1].

We crystallized recombinant chicken muscle CapZ and collected diffraction data at beamlines **BL45XU-PX** and **BL44B2**, and determined the structure at 2.1 Å resolution [2]. The obtained structures of  $\alpha$  and  $\beta$  subunits of CapZ show striking resemblance, although they have no significant homology in the amino acid sequences. The two subunits assemble

tightly with a pseudo two-fold symmetrical geometry through an extended hydrophobic surface.

Previous mutagenesis experiments indicated that the C-terminus of each subunit may be responsible for actin binding. Our crystal structure (Fig. 2) showed that 33 residues of the C-terminus of the  $\beta$ -subunit have no specific interaction with the main body of the protein, whereas 27 residues of the C-terminus of the  $\alpha$ -subunit may have weak interactions with the main body. Each of the C-terminus contains a stretch of an amphipathic  $\alpha$ -helix and is “anchored” by a conserved arginine residue, Arg-259 for  $\alpha$ -tentacle and Arg-244 for  $\beta$ -tentacle, to the main body.

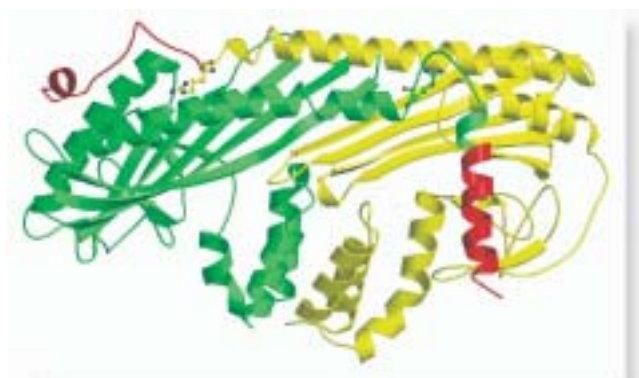


Fig. 2. A ribbon representation of the crystal structure of chicken muscle CapZ. The C-terminus (red) of each subunit,  $\alpha$ - (yellow) and  $\beta$ - (green) subunits, of the hetero-dimeric molecule is responsible for actin binding. Arg-259 ( $\alpha$ ) and Arg244 ( $\beta$ ), in ball-and-stick representation, may function as pivots.



Fig. 1. Capping protein (CP) in the actin dynamics, which is a molecular movement driven by the polymerization and depolymerization of actin. The actin dynamics plays crucial roles in many cellular functions. CP is indispensable for this dynamics; it reduces the number of growing ends at the leading edge, thereby increasing the rate of forward movement [6].

On the basis of these observations, we proposed a “tentacle” model for the actin capping of CapZ. Actin binding is made possible predominantly by a pair of tentacles, which may protrude from the main body of the protein (Fig. 3). It is also postulated that the hydrophobic side of the  $\alpha$ -helix within each tentacle functions as an interface to the surface of actin subunits, whereas the manner of binding of a pair of tentacles to the B-end of an actin filament remains elusive, largely due to the lack of information on the CapZ-binding site on the actin filament.

Immediately after obtaining the crystal structure, we started systematic mutation

and deletion experiments of chicken muscle CapZ [3] and yeast CP [4] in collaboration with John Cooper's laboratory in St. Louis. We believe that mutagenesis experiments should be useful only after the crystal structure is obtained.

In both chicken muscle CapZ and yeast CP, mutants lacking both tentacles did not show any capping activity, indicating that the pair of tentacles are the major actin-binding regions. CapZ (CP) with only the  $\alpha$ -tentacle did show capping activity with a reduced affinity (by a factor of 400 in chicken CapZ, 7 in yeast CP), mainly due to the increased off-rate. On the other hand, CapZ (CP) with only the  $\beta$ -tentacle did not show a significant capping activity. These data indicate that the pair of tentacles are functionally independent and nonequivalent to each other.

An interesting information about the posture of the  $\alpha$ -tentacle came from the study of interaction between CapZ and another protein S100B. Although the peptide corresponding to the  $\alpha$ -tentacle specifically interacts with S100B, CapZ (with an intact  $\alpha$ -tentacle) does not [5]. This indicates that, in solution, the  $\alpha$ -tentacle does not protrude out from the main body of the protein, rather it lies down on the surface of the protein.

Our understanding on how CapZ caps the B-end of an actin filament is far from complete. We do not know in which posture CapZ binds the B-end, or whether each tentacle interacts with one actin monomer or two. More importantly, we should keep in mind that an actin filament, particularly its B-end, is dynamic. Our preliminary computer simulation (T. Oda & Y. Maéda) based on our atomic model of an actin filament indicates that the separation between two end monomers must be always fluctuating. This may be consistent with the fact that, with CapZ, the main body is well built and hydrophobic interactions between the two subunits are extremely tight, being suitable to bear the tearing force.

Our understanding of functional aspects of CapZ is also not complete. We do not know yet how CapZ is removed from the B-end. We also do not know much about the mechanism by which CapZ targets the actin filament in a muscle cell, that is, the nature of the interaction between CapZ and proteins to be targeted.

It is, however, clear that our elucidation of the crystal structure has opened up a new era in the study of the capping mechanism of CapZ at the B-end of an actin filament.

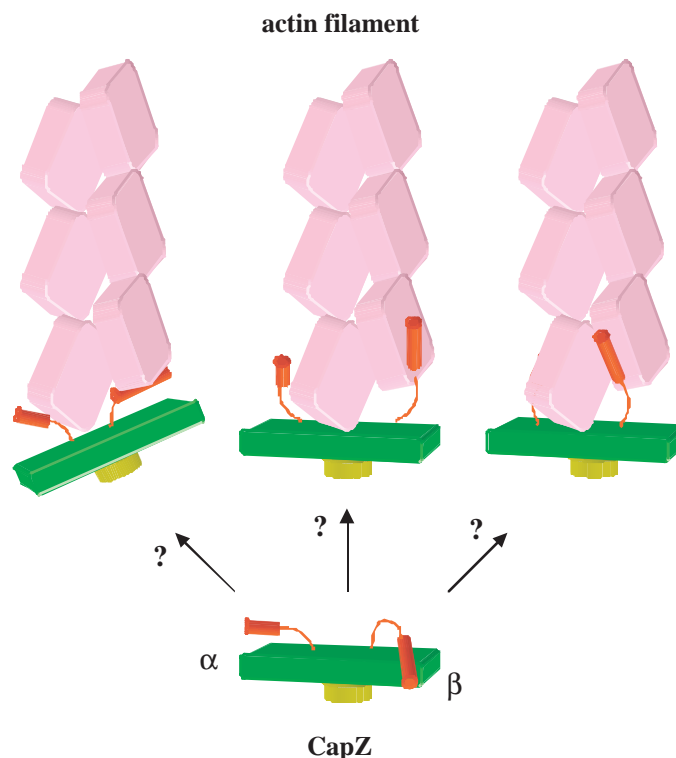


Fig. 3. Schematic drawings of the possible modes of binding of the capping protein to the actin filament (speculative models) [2].

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### References

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