Actin Molecule Becomes a Flat Conformation by Polymerization

"Movement" or "Motility" is a fundamental activity in life at multiple scales from body to molecules. In eukaryote cells, there are two kinds of motility system. One is a system composed of motor proteins and rails. The motor proteins move along the rail that is formed by polymerizable proteins, while hydrolyzing ATP, a source of chemical energy. A characteristic of the systems is a high efficiency to convert chemical energy to motility or mechanical work; it exceeds 80% in muscle. The other is the system driven by a polymerization and depolymerization cycle of polymerizable proteins. Actin and tubulin are classified in this category called "cytomotive protein." Similar motility systems are also observed in prokaryote cells, whereas no motor proteins have been found. Therefore, this is an evolutionally primitive system for motility.

Actin monomer (G-actin) bound with ATP is incorporated into actin filament (F-actin), and thereby the actin molecule hydrolyzes the ATP into ADP and phosphate, followed by the release of the cleaved phosphate, and finally, the actin molecule bound with ADP dissociates from the F-actin. The dissociated G-actin exchanges the bound nucleotide from ADP to ATP. Two ends of the F-actin are called the barbed-end and the pointed-end. Owing to the actin ATPase, the critical actin concentration for polymerization is low at the barbed-end while it is high at the pointed-end. Thus, under the physiologically steady condition, the elongation of F-actin mainly occurs at the barbed-end while shrinking is dominant at the pointed-end. The unidirectional polymerization process is a driving force for motility.

To understand the mechanisms of their functions, the structures of G-actin and F-actin are essential. The crystal structure of G-actin was solved in 1990 [1], and an atomic model for the F-actin structure was also proposed, by piling up the G-actin crystal structure according to its helical symmetry [2]. The model accounted for the results of many biochemical experiments. However, the model without any conformational changes does not account for actin functions accompanied by polymerization, such as the activation of the actin ATPase. The conformational changes remained enigmatic.

X-ray fiber diffraction method is suitable for structural analysis of rod-like macromolecule complexes with a helical symmetry. An X-ray fiber diffraction pattern is essentially a section through the cylindrically averaged diffraction pattern from a single fibrous molecule. The cylindrical averaging leads to a significant loss of information. High resolution is essential to obtain a clear answer. To achieve high resolution, we combined several technical advances: we controlled the filament length by adding gelsolin, prepared well-oriented sols of F-actin using an 18 tesla superconducting magnet, and recorded X-ray fiber diffraction patterns to 3 Å at beamlines **BL40B2**, **BL41XU** and **BL45XU**. We created a model of F-actin by using the X-ray fiber diffraction intensities, obtained from well-oriented sols of rabbit skeletal muscle F-actin to 3.3 Å in the radial direction and 5.6 Å along the equator, by altering the crystal structure of G-actin, using the normal modes of actin and a molecular dynamics simulation, while monitoring the R-factor-non-fit, which is equivalent to the free R-factor.

Actin has a nucleotide-binding cleft enclosed by two major domains (Fig. 1(a)). In almost all actin crystal structures, the cleft is closed and the two major domains are in a propeller-like twist with each other.

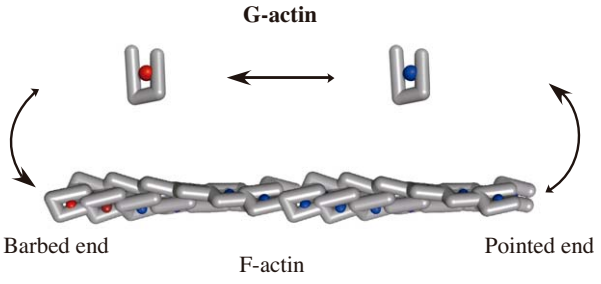

Fig. 1. A polymerization and depolymerization cycle of actin. The cycle is coupled with the actin ATPase. The red sphere represents ATP and the blue sphere represents ADP. Actin has two states: a monomeric G-actin and a polymeric F-actin [4].

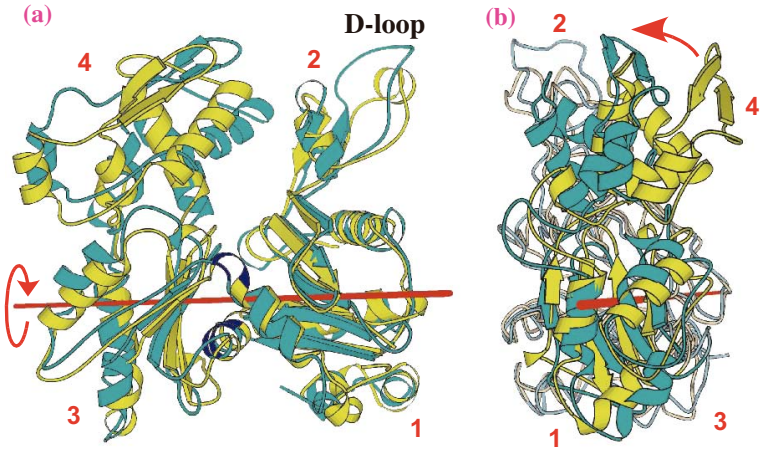

Fig. 2. Comparisons between G-actin (pdb code: 1J6Z: yellow) and F-actin subunit (cyan). **(a)** front view. **(b)** side view. The red line is a rotation axis. The red numbers represent subdomain numbers [5].

This is characteristic of the G-actin conformation (yellow in Figs. 1(a), 1(b)). In our F-actin model, we found a previously unknown flat conformation, in which the cleft remains closed and the two domains are untwisted (cyan in Figs. 1(a), 1(b)). The two conformations are related by a 20° rotation of the two major domains around the axis passing along the front of subdomain 1 and the side of subdomain 3. As a result, the F-actin subunit shows a flat conformation. The overall structure of our F-actin model is shown in Fig. 3(a). The contacts between the actin subunits are shown in Figs. 2(b) and 2(c). A comparison of our F-actin model with the non-helical arrangement of normal G-actin observed in the crystal [3] suggests that the flattening generates extensive intrastrand and interstrand connections.

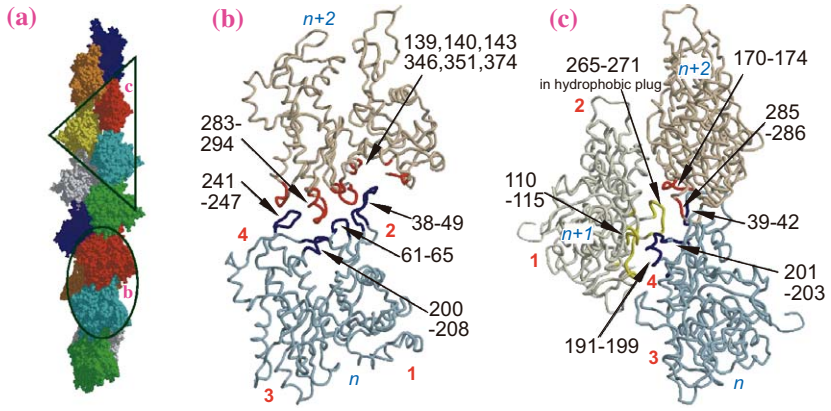

Fig. 3. Overall structure of F-actin. **(a)** Descriptions of F-actin using 13 subunits. In the experiment, the average filament length was controlled to be *ca.* 1 μm (*ca.* 400 subunits). **(b, c)** Contact sites between subunits within F-actin [5].

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References

[1] W. Kabsch *et al.*: Nature **347** (1990) 37.
[2] K.C. Holmes *et al.*: Nature **347** (1990) 44.
[3] T. Otomo *et al.*: Nature **433** (2005) 488.
[4] T. Oda: Tanpakushitsu Kakusan Koso **54** (2009) 1864 (in Japanese).
[5] *T. Oda, M. Iwasa, T. Aihara, Y. Maéda and A. Narita: Nature* ***457*** *(2009) 441.*