Cell

Structural Basis for Actin Assembly, Activation of ATP Hydrolysis, and Delayed Phosphate Release

Kenji Murakami,[1,6] Takuo Yasunaga,[3] Taro Q.P. Noguchi,[4] Yuki Gomibuchi,[1] Kien X. Ngo,[4] Taro Q.P. Uyeda,[4] and Takeyuki Wakabayashi[1,2,5,*]

[1]Department of Biosciences, School of Science and Engineering
[2]Department of Judo Therapy, Faculty of Medical Technology
Teikyo University, Toyosatodai 1-1, Utsunomiya 320-8551, Japan
[3]Department of Bioscience and Bioinformatics, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology, Ooaza-kawazu 680-4, Lizuka, Fukuoka 820-850, Japan
[4]Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology, AIST Tsukuba Central 4, 1-1-1 Highashi, Tsukuba, Ibaraki 305-8562, Japan
[5]Department of Physics, School of Science, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan
[6]Present address: Department of Structural Biology, Stanford University, Stanford, CA 94305, USA
*Correspondence: wakabayashi@nasu.bio.teikyo-u.ac.jp
DOI 10.1016/j.cell.2010.09.034

SUMMARY

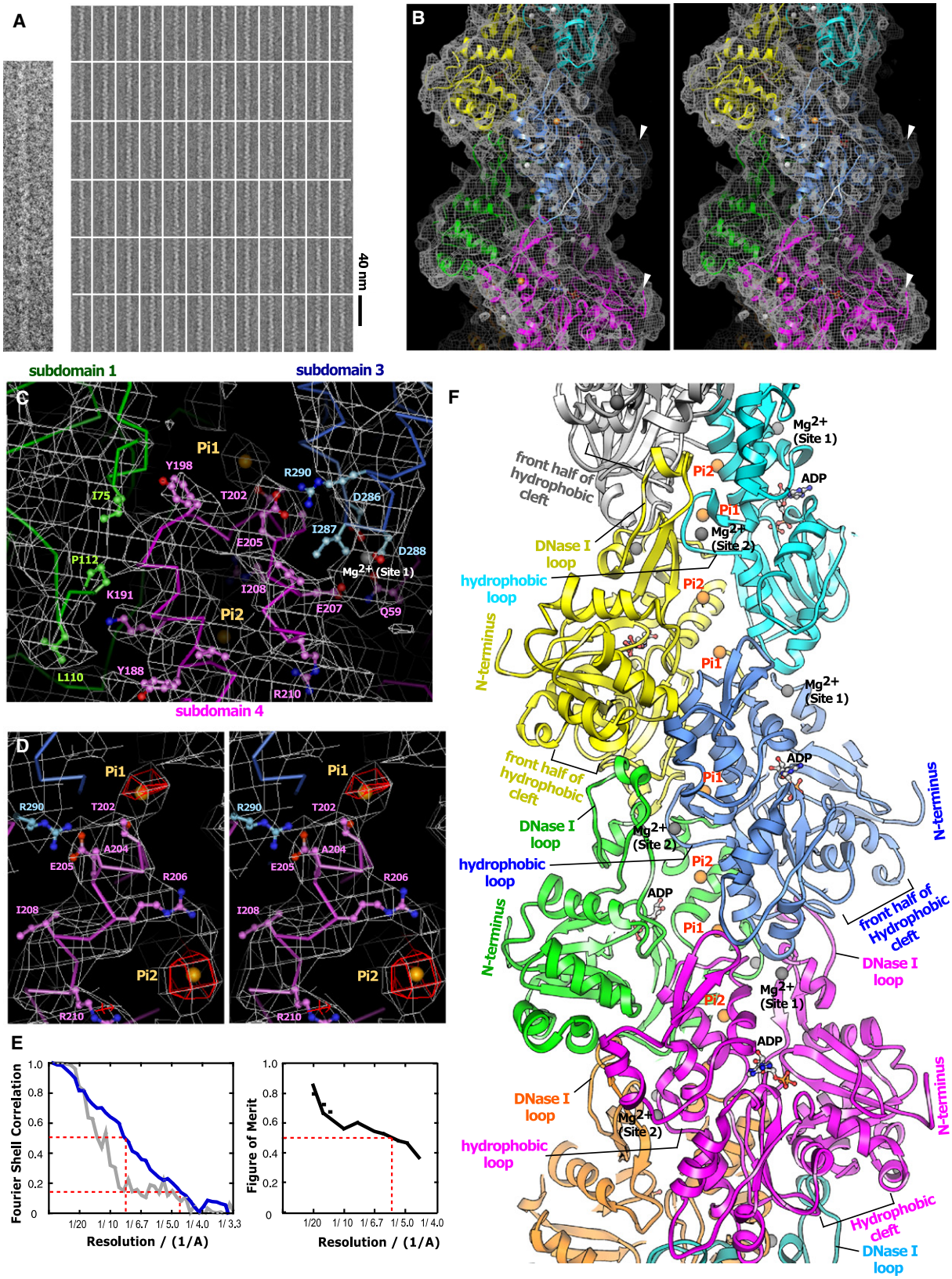
Assembled actin filaments support cellular signaling, intracellular trafficking, and cytokinesis. ATP hydrolysis triggered by actin assembly provides the structural cues for filament turnover in vivo. Here, we present the cryo-electron microscopic (cryo-EM) structure of filamentous actin (F-actin) in the presence of phosphate, with the visualization of some α-helical backbones and large side chains. A complete atomic model based on the EM map identified intermolecular interactions mediated by bound magnesium and phosphate ions. Comparison of the F-actin model with G-actin monomer crystal structures reveals a critical role for bending of the conserved proline-rich loop in triggering phosphate release following ATP hydrolysis. Crystal structures of G-actin show that mutations in this loop trap the catalytic site in two intermediate states of the ATPase cycle. The combined structural information allows us to propose a detailed molecular mechanism for the biochemical events, including actin polymerization and ATPase activation, critical for actin filament dynamics.

INTRODUCTION

The actin-filament system is required in almost all cytoplasmic processes, including cell adhesion, motility, cellular signaling, intracellular trafficking, and cytokinesis. Although stable actin filaments (F-actin) are necessary during muscle contraction, the active turnover of filaments is required in many cell functions. Actin has two major domains separated by a nucleotide-binding cleft (Kabsch et al., 1990). The outer domain is divided into subdomains 1 and 2 and the inner domain into subdomains 3 and 4. All of the subdomains interact with the bound nucleotide. ATP is hydrolyzed at the rate of 1/3.3 s^{-1} following the elongation of filaments at the growing end of filaments (Blanchoin and Pollard, 2002), whereas the phosphate release is 100 times slower (Carlier and Pantaloni, 1986). As a result, newly polymerized filaments consist of stable ADP-Pi actin (abbreviated as F-ADP-Pi), whereas the older filaments contain mainly ADP actin (F-ADP), which disassembles more rapidly (Carlier and Pantaloni, 1986). Under physiological conditions, inorganic phosphate (Pi) binds to F-actin and reduces the critical concentration for polymerization (Rickard and Sheterline, 1986; Fujiwara et al., 2007). Actin dynamics also depends on the identity of the bound divalent cation, physiologically Mg^{2+}, associated with the bound nucleotide (Carlier et al., 1986).

Although a vast amount of biochemical data has been accumulated, the quest for a definitive and detailed molecular mechanism of the polymerization of monomeric actin (G-actin) to filamentous actin (F-actin) has been hampered by the inherent flexibility of actin filament. The flexibility has not allowed an atomic structure of F-actin to be determined. More than 50 atomic structures of G-actin bound with ATP or ADP have been determined since 1990 (Kabsch et al., 1990), but F-actin has been visualized to relatively moderate resolution either by three-dimensional (3D) image reconstruction from electron micrographs (Belmont et al., 1999) or modeling based on X-ray fiber diagrams (Holmes et al., 1990; Lorenz et al., 1993). The inherent flexibility of actin filaments hampers determination of atomic structure.

Recently, a new model of F-actin based on improved X-ray fiber diffraction analysis was reported (Oda et al., 2009). Oda et al. proposed that outer-domain movement upon assembly flattens the actin molecule in the polymer, similar to the case of the bacterial actin homolog MreB (van den Ent et al., 2001),


A
40 nm
B
subdomain 1
subdomain 3
C
Pi1
Y198
R290
T202
D286
I75
I287
E205
D288
P112
Mg2+ (Site 1)
K191
I208
Pi2
E207
Q59
L110
Y188
R210
subdomain 4
D
Pi1
R290
T202
A204
E205
R206
I208
Pi2
R210
E
Fourier Shell Correlation
Resolution / (1/A)
Figure of Merit
Resolution / (1/A)
F
Mg2+ (Site 1)
front half of hydrophobic cleft
Pi2
ADP
Pi1
Mg2+ (Site 2)
DNase I loop
hydrophobic loop
N-terminus
front half of Hydrophobic cleft
Hydrophobic cleft