FEBS Letters 589 (2015) 3037–3043

FEBS Letters

journal homepage: www.FEBSLetters.org

Biophysical characterization of the interaction between FAAP20-UBZ4 domain and Rev1-BRCT domain

Kyungeun Lim [a], Mi-Kyung Lee [b], Phuong T.M. Duong [a], Dinan Liu [a], Sieun Sung [a], Byong-Seok Choi [a,*]

[a] Department of Chemistry, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Structural biology & Nanopore laboratory, Functional Genomics Research Center, KRIBB, 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

ARTICLE INFO

Article history:
Received 22 May 2015
Revised 5 August 2015
Accepted 17 August 2015
Available online 28 August 2015

Edited by Kazuhiro Iwai

Keywords:
Fanconi anemia pathway
Interstrand cross-link
Fanconi anemia-associated protein 20
Rev1
Ubiquitin
Nuclear magnetic resonance

ABSTRACT

FAAP20 (Fanconi anemia-associated protein 20) is a subunit of the Fanconi anemia (FA) core complex that repairs interstrand cross-links. To understand the molecular basis for the FA core complex-mediated recruitment of Rev1 to the DNA lesion, we characterized the interactions among FAAP20-UBZ4, Rev1-BRCT, and ubiquitin using NMR. We found that FAAP20-UBZ4 binds not only ubiquitin but also Rev1-BRCT. Mapping the protein–protein interactions showed that FAAP20-UBZ4 has distinct binding surfaces for ubiquitin and Rev1-BRCT. In addition, the chemical exchange patterns indicated that the interaction between FAAP20-UBZ4 and ubiquitin might enhance the binding affinity between FAAP20-UBZ4 and Rev1-BRCT. These results provide new insight into the Rev1 recognition mechanism by FAAP20.

1. Introduction

Fanconi anemia (FA) is a genetic disease characterized by acute myelogenous leukemia, bone marrow failure, and congenital defects such as short stature and abnormalities of the skin, arms, head, eyes, kidneys, and ears [1,2]. This disorder is caused by a genetic defect in a group of proteins, known as FA proteins, in charge of DNA interstrand cross-link (ICL) repair. On account of the defect in DNA repair, cells derived from FA patients are hypersensitive to DNA cross-linking agents, such as mitomycin C (MMC) or diepoxybutane (DEB), and present with multiple chromatid breaks and radial structures [3]. On the other hand, in normal cells, DNA ICLs block transcription and replication by inhibiting DNA strand separation and require DNA repair for chromosomal stability.

The FA pathway is responsible for DNA ICL repair, and at least 16 FA proteins are involved and cooperate in this pathway. In response to DNA damage, the FA core complex, which is composed of at least 8 FA proteins (FANCA/B/C/E/F/G/L/M), is recruited to the ICL region. As an E3 ligase, the FA core complex monoubiquitinates FANCD2 and FANCI, and the monoubiquitinated FANCD2–FANCI heterodimer recruits a nuclease for nucleolytic incision near the ICL. Then translesion synthesis (TLS) allows the DNA replication machinery to replicate past the DNA lesion, creating a nascent strand for homologous recombination (HR). The double-strand break (DSB), created by the incision process, is repaired by HR and the remaining adducts, cross-linked nucleotides, are removed by nucleotide excision repair (NER) [4,5].

TLS is one of several DNA repair mechanisms that tolerates error-prone DNA repair, resulting in residual point mutations. TLS requires specialized DNA polymerases, namely η, ι, κ, and Rev1 in the Y-family and polymerase ζ in the B-family. The TLS polymerases feature relatively poor processivity and low fidelity in comparison to the regular DNA polymerases. When a regular DNA polymerase encounters a stalled replication fork, one of the TLS polymerases replaces it according to the type of DNA damage [6,7].

A recent study reported the regulation of TLS by FA core complex-mediated recruitment of Rev1, suggesting that the FA

Abbreviations: FA, Fanconi anemia; ICL, interstrand cross-link; DSB, double-strand break; HR, homologous recombination; MMC, mitomycin C; DEB, diepoxybutane; TLS, translesion synthesis; NER, nucleotide excision repair; FAAP20, Fanconi anemia-associated protein 20; UBZ4, ubiquitin-binding zinc finger 4; BRCT, BRCA1 C-terminus; PBS, phosphate-buffered saline; ITC, isothermal titration calorimetry; TEV, tobacco etch virus

Authors contributions: K.L. and D.L. designed experiments. K.L., M.L., P.T.D., D.L. and S.S. performed experiments. K.L., M.L. and B.C. analyzed experiments. K.L. and B.C. wrote the manuscript. B.C. directed the project.

* Corresponding author. Fax: +82 42 350 5828.
E-mail address: byongseok.choi@kaist.ac.kr (B.-S. Choi).

http://dx.doi.org/10.1016/j.febslet.2015.08.021
0014-5793/

core complex, either directly or indirectly, regulates the BRCT domain-mediated loading of Rev1 onto replication forks [8]. Furthermore, Fanconi anemia-associated protein 20 (FAAP20) was recently identified as an integral subunit of the multisubunit FA core complex [9–12]. FAAP20 maintains the integrity of the FA core complex through an interaction with FANCA using FAAP20's N-terminus (Fig. 1A). The C-terminus of FAAP20 contains a ubiquitin-binding zinc finger 4 (UBZ4) domain (Fig. 1A), which was identified to interact with Rev1 for promoting TLS [9].

To understand the molecular basis for FA core complex-mediated recruitment of Rev1, we characterized the interactions among FAAP20-UBZ4, Rev1-BRCT, and ubiquitin by nuclear magnetic resonance (NMR) and isothermal titration calorimetry (ITC) experiments. In this study, we identified that FAAP20-UBZ4 interact with ubiquitin and Rev1-BRCT through distinct regions and suggested a mechanism by which FAAP20 recognizes Rev1 during ICL repair.

2. Materials and methods

2.1. Molecular cloning

The human FAAP20-UBZ4 domain (residues 103–180), FAAP20-UBZ4 domain (residues 133–180) and Rev1-BRCT domain (residues 44–132) were cloned into the modified pGEX 4T vector (GE-Healthcare) containing an N-terminal GST tag and a tobacco etch virus (TEV) protease site. The human ubiquitin (residues 1–76) was cloned into the pET15b vector (Novagen) containing an N-terminal His_6 tag and a thrombin protease site.

2.2. Protein expression and purification

The expression plasmids were transformed into *Escherichia coli* BL21(DE3) cells. Following the addition of 200 μM $ZnCl_2$ at 0.4 OD_{600}, the expression of both GST-fused FAAP20-UBZ4 constructs was induced by addition of 0.4 mM IPTG at 0.6 OD_{600}. After 5 h further incubation at 30 °C, the harvested cells were suspended in phosphate-buffered saline (PBS) (10 mM Na_2HPO_4, 140 mM NaCl, 1.8 mM KH_2PO_4, pH 7.5) and lysed by sonication. Cell debris was removed by centrifugation at 15 000 rpm for 1 h and the supernatant was loaded onto a GST column (Clontech). After washing with PBS, the protein was eluted by elution buffer (50 mM Tris–HCl, pH 8.0, 33 mM glutathione) prepared freshly. After the protein was cleaved with TEV protease for 16 h at 4 °C, it was dialyzed using Q-buffer (50 mM sodium phosphate, 50 mM NaCl, 2 mM DTT, pH 6.4) and then loaded onto a Q HP column (GE-Healthcare) automated by an AKTA machine to separate the target protein from the GST tag and TEV protease. The peak fraction was eluted by a 50–1000 mM linear gradient of NaCl.

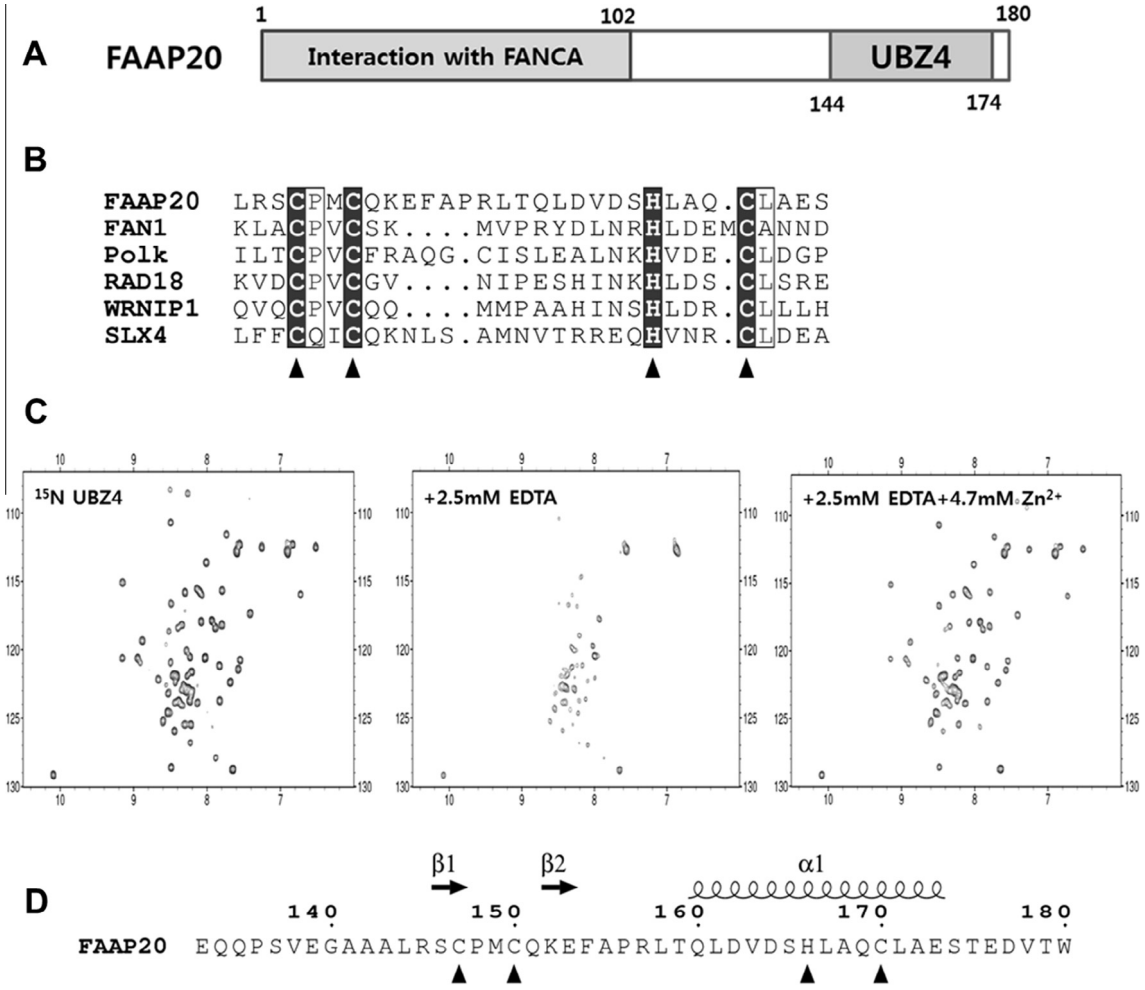

Fig. 1. FAAP20 contains a ubiquitin-binding zinc finger 4 (UBZ4) domain in its C-terminus. (A) Schematic diagram of full-length FAAP20. (B) Sequence alignment of the FAAP20-UBZ4 domain (residues 144–174) with other UBZ4 domains using ESPript 3.0 [18]. The conserved Cys and His residues are shaded in black and indicated by triangles (▲). Open boxes indicate highly similar residues greater than a similarity score's value (0.7). (C) 2D ^{1}H, ^{15}N-HSQC spectra of the FAAP20-UBZ4 domain (residues 103–180) alone and in the presence of EDTA and EDTA plus Zn^{2+}. (D) The human FAAP20 sequence (residues 133–180) used in this study, with secondary structure elements indicated (PDB ID: 2MUQ). α = α-helix, β = β-strand. The residues coordinating Zn^{2+} are indicated by triangles (▲).