DNA Repair 31 (2015) 80–90

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Association of the Rad9–Rad1–Hus1 checkpoint clamp with MYH DNA glycosylase and DNA

Bor-Jang Hwang[a], Jin Jin[a], Randall Gunther[a], Amrita Madabushi[a,b], Guoli Shi[a,c], Gerald M. Wilson[a,d], A.-Lien Lu[a,d,*]

[a] Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201, United States
[b] Department of Natural and Physical Sciences, Life Sciences Institute; Baltimore City Community College, Baltimore, MD 21201, United States
[c] University of Maryland School of Nursing, Baltimore, MD 21201, United States
[d] Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, United States

A R T I C L E I N F O

Article history:
Received 11 December 2014
Received in revised form 4 May 2015
Accepted 8 May 2015
Available online 16 May 2015

Keywords:
DNA repair
DNA binding protein
DNA damage response
DNA glycosylase
Genomic instability

A B S T R A C T

Cell cycle checkpoints provide surveillance mechanisms to activate the DNA damage response, thus preserving genomic integrity. The heterotrimeric Rad9–Rad1–Hus1 (9–1–1) clamp is a DNA damage response sensor and can be loaded onto DNA. 9–1–1 is involved in base excision repair (BER) by interacting with nearly every enzyme in BER. Here, we show that individual 9–1–1 components play distinct roles in BER directed by MYH DNA glycosylase. Analyses of Hus1 deletion mutants revealed that the interdomain connecting loop (residues 134–155) is a key determinant of MYH binding. Both the N-(residues 1–146) and C-terminal (residues 147–280) halves of Hus1, which share structural similarity, can interact with and stimulate MYH. The $Hus1^{K136A}$ mutant retains physical interaction with MYH but cannot stimulate MYH glycosylase activity. The N-terminal domain, but not the C-terminal half of Hus1 can also bind DNA with moderate affinity. Intact Rad9 expressed in bacteria binds to and stimulates MYH weakly. However, $Rad9^{1-266}$ (C-terminal truncated Rad9) can stimulate MYH activity and bind DNA with high affinity, close to that displayed by heterotrimeric 9^{1-266}–1–1 complexes. Conversely, Rad1 has minimal roles in stimulating MYH activity or binding to DNA. Finally, we show that preferential recruitment of 9^{1-266}–1–1 to 5′-recessed DNA substrates is an intrinsic property of this complex and is dependent on complex formation. Together, our findings provide a mechanistic rationale for unique contributions by individual 9–1–1 subunits to MYH-directed BER based on subunit asymmetry in protein–protein interactions and DNA binding events.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Cell cycle checkpoints provide surveillance mechanisms to activate the DNA damage response (DDR), thus preserving genomic integrity [1,2]. Activation of DDR leads to cell cycle arrest (which allows time for DNA repair) and enhances DNA repair. When DNA damage is extreme, apoptosis is triggered. The checkpoint system includes an array of proteins that function as sensors, transducers, and effectors [3–6]. The heterotrimeric Rad9/Rad1/Hus1 (9–1–1) complex is a DDR sensor [7,8] and is loaded onto DNA by the Rad17-RFC_{2-5} clamp loader [9–12]. 9–1–1 is essential for embryonic development, genomic stability, and telomere integrity [13–16]. Besides serving as a damage sensor [17], 9–1–1 is involved in many DNA metabolisms [14] including base excision repair (BER) (reviewed in [18]). Remarkably, 9–1–1 interacts with nearly every enzyme in BER and is proposed to constitute a platform to coordinate BER. Many BER proteins interact selectively with specific subunit(s) of 9–1–1 [19–21].

The first step in BER is carried out by a DNA glycosylase, which cleaves damaged or mismatched bases. MYH (also called MUTYH) DNA glycosylase excises adenine when it is mispaired with 8-oxo-7,8-dihydroguanine (G^0) or guanine and thus reduces G:C to T:A

Abbreviations: 9–1–1, Rad9, Rad1, and Hus1 heterotrimer complex; AP, apurinic/apyrimidinic; APE1, AP endonuclease; BER, base excision repair; DDR, DNA damage response; DTT, dithiothreitol; ECL, enhanced chemiluminescence; EMSA, electrophoresis mobility shift assay; G^0 or 8-oxoG, 7,8-dihydro-8-oxo-guanine; GST, glutathione-*S*-transferase; h, human; h coefficient, Hill (cooperative) coefficient; IDC, interdomain connector; IDCL, interdomain connecting loop; m, mouse; MYH, MutY homolog; $[P]_{1/2}$, protein concentration yielding half-maximal DNA binding; PCNA, proliferating cell nuclear antigen; PCR, polymerase chain reaction; $Rad9^{1-266}$, hRad9 containing residues 1–266 of 391; RFC, replication factor C; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Sp, *Schizosaccharomyces pombe*; TBE, tri-borate-EDTA buffer.

* Corresponding author at: Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201, United States. Tel.: +1 410 706 4356; fax: +1 410 706 8297.
E-mail address: alu-chang@som.umaryland.edu (A.-L. Lu).

http://dx.doi.org/10.1016/j.dnarep.2015.05.004
1568-7864/© 2015 Elsevier B.V. All rights reserved.

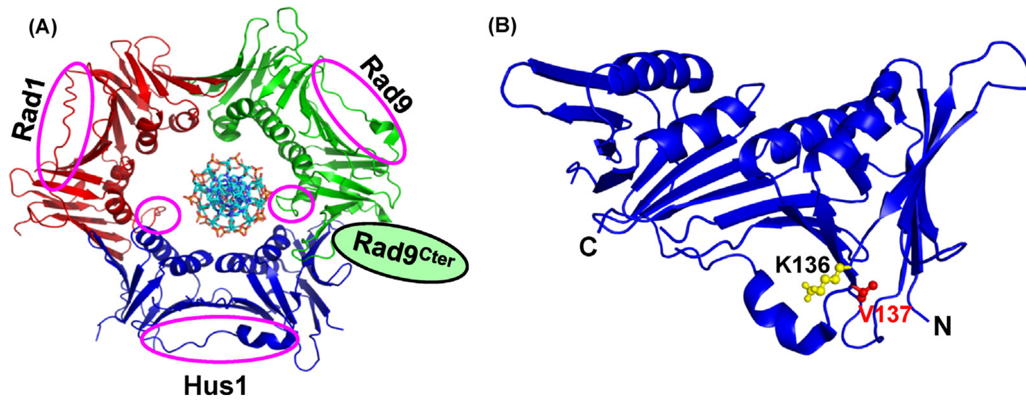

Fig. 1. (A) Structure of the human 9–1–1 complex. The crystal structure 9^{1-272}–1–1 is shown (RCSB codes:3A1J) [30]. The structure of the extreme C-terminal domain of Rad9 ($Rad9^{Cter}$) has not been determined. The key differences among the three subunits are circled in red. The DNA in the channel of the 9–1–1 ring is tilted toward Rad9 and the N-terminal domain of Hus1 based on the data from this paper. (B) Structure of Hus1 showing the locations of K136 and V137 based on the structure of 9^{1-272}–Rad1–Hus1 (RCSB codes:3A1J) [30]. N and C indicate N- and C-termini, respectively.

mutations [22–24]. The resulting apurinic/apyrimidinic (AP) site is processed by AP-endonuclease 1 (APE1), allowing the downstream BER enzymes to complete the DNA repair process. Mutations in the human *MYH* (*hMYH*) gene can lead to colorectal cancer (as in MYH-associated polyposis or MAP) [25], while APE1 is essential for cell viability [26]. MYH contains unique motifs that mediate interactions with partner proteins involved in DNA replication, mismatch repair, and DDR (reviewed in [22,23]). We have shown that the interdomain connector (IDC) located between the N- and C-terminal domains of hMYH is uniquely oriented [27] to interact with Hus1 [21] and APE1 [28].

The ring structure of 9–1–1 [29–31] is remarkably similar to that of the proliferating cell nuclear antigen (PCNA) [32–34]. Each 9–1–1 subunit folds into two globular domains linked by an interdomain connecting loop (IDCL) (Fig. 1A). According to the PCNA–DNA structure [35], the 9–1–1 ring is supposed to encircle double-stranded DNA [30]. Although the three subunits of the 9–1–1 complex are structurally similar, they exhibit key differences. These differences are most pronounced in the IDCLs between their N- and C-terminal domains [29–31]. These structural distinctions between 9–1–1 components have been suggested to dictate protein-binding specificity for individual subunits. For example, hMYH and hAPE1 bind preferentially to the Hus1 subunit [20,21]. Functionally, the Hus1 subunit alone can stimulate MYH activity [21]. In this paper, we constructed a panel of Hus1 mutant proteins to identify domains required for binding MYH, stimulating MYH glycosylase activity, and binding DNA. Subsequently we tested the roles of other 9–1–1 components in MYH activation and DNA binding. This systematic and quantitative biochemical strategy revealed key differences in the ability of 9–1–1 subunits to bind DNA and functionally interact with MYH, thus supporting a model whereby each subunit of 9–1–1 plays a distinct and directed role in BER.

2. Materials and methods

2.1. Glutathione-S-transferase (GST)-tagged Hus1 protein constructs

The plasmid pGEX-3X-hHus1 containing GST-tagged hHus1 was obtained from Dr. A.E. Tomkinson at the University of New Mexico. GST fusions incorporating hHus1 deletion constructs were made by polymerase chain reaction (PCR) using primers listed in Table S1 in the Supplementary material. The PCR products were digested with BamHI and SalI and ligated into the BamHI-XhoI-digested pGEX-4T-2 vector (GE Healthcare). The K136A and V137A mutants of the *hHus1* gene were constructed by QuickChange site-directed mutagenesis (Stratagene) using pGEX-3X-hHus1 plasmid as a template and primers listed in Table S1 in the Supplementary material.

2.2. Cloning and purification of His-tagged hHus1 and deletion constructs

Plasmid pET21a-hHus1 containing full-length wild-type *hHus1* cDNA has been described [21]. PCR products containing truncated *hHus1* cDNA fragments were digested with BamHI and SalI (as described Section 2.1) and ligated into BamHI-XhoI-digested pET21a. The K136A and V137A mutants of the *hHus1* gene were constructed by QuickChange site-directed mutagenesis (Stratagene) using pET21-hHus1 plasmid as a template as described for GST clones.

Escherichia coli Rosetta cells (Novagen) harboring the hHus1-His expression plasmid were grown and induced as described [21]. The hHus1-His proteins were purified over a Ni-NTA resin (Qiagen) and a 1 ml Hitrap Heparin column (GE Healthcare) as described [21]. Fractions containing the majority of hHus1-His proteins were pooled, divided into small aliquots, and stored at −80 °C. The purified proteins were analyzed by polyacrylamide gel electrophoresis containing SDS (SDS-PAGE) (Fig. 2), with protein concentrations determined by the Bradford method [36].

2.3. Cloning and purification of human $hRad9^{1-266}$–Rad1–Hus1

The cDNAs of *hRad9* and *hRad1* were amplified by PCR from pGEX-4T3-hRad9 and pGEX-3X-hRad1 plasmids, respectively (both from Dr. A.E. Tomkinson) using primers listed in Table S1 in the Supplementary material. The *hRad1* gene was cloned between the BamHI and SalI sites of pACYCDuet-1 (EMD Biosciences) to obtain the clone pACYCD-hRad1. The *hHus1* cDNA was then inserted into the second cassette of the pACYCD-hRad1 plasmid using BglII and XhoI sites to obtain pACYCDuet-hRad1-hHus1. A *hRad9* cDNA fragment encoding amino acid residues 1–266 were cloned between the BamHI and SalI sites of pETDuet-1 to obtain pETD-$hRad9^{1-266}$. Both hRad1 and $hRad9^{1-266}$ proteins contained an N-terminal His tag while hHus1 was tagged with a C-terminal S-tag.

E. coli BL21Star/DE3 harboring both pACYCDuet-hRad1-hHus1 and pETD-$hRad9^{1-266}$, were grown and induced as described [21]. The 9^{1-266}–1–1 complex was first purified over a Ni-NTA resin

Download English Version:

https://daneshyari.com/en/article/1980031

Download Persian Version:

https://daneshyari.com/article/1980031

Daneshyari.com