Mutation Research 763 (2015) 306–320

Contents lists available at ScienceDirect

Mutation Research/Reviews in Mutation Research

journal homepage: www.elsevier.com/locate/reviewsmr
Community address: www.elsevier.com/locate/mutres

Review

Overview of xeroderma pigmentosum proteins architecture, mutations and post-translational modifications

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ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form 8 December 2014
Accepted 9 December 2014
Available online 16 December 2014

Keywords:
Xeroderma pigmentosum
Protein structure
TC-NER
GG-NER
DNA repair
Post-translational modifications

ABSTRACT

The xeroderma pigmentosum complementation group proteins (XPs), which include XPA through XPG, play a critical role in coordinating and promoting global genome and transcription-coupled nucleotide excision repair (GG-NER and TC-NER, respectively) pathways in eukaryotic cells. GG-NER and TC-NER are both required for the repair of bulky DNA lesions, such as those induced by UV radiation. Mutations in genes that encode XPs lead to the clinical condition xeroderma pigmentosum (XP). Although the roles of XPs in the GG-NER/TC-NER subpathways have been extensively studied, complete knowledge of their three-dimensional structure is only beginning to emerge. Hence, this review aims to summarize the current knowledge of mapped mutations and other structural information on XP proteins that influence their function and protein–protein interactions. We also review the possible post-translational modifications for each protein and the impact of these modifications on XP protein functions.

Contents

1. Introduction

Exposure to a range of environmental hazards, such as ultraviolet (UV) light and various toxic pollutants, can induce the formation of bulky DNA lesions. Among these bulky DNA lesions, both 6-4 photoproducts and cyclobutane pyrimidine dimers (CPDs) represent the most common UV-induced nucleotide damage. 6-4 photoproducts are formed from the reaction between

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http://dx.doi.org/10.1016/j.mrrev.2014.12.002
1383-5742/

the C5–C6 bond of a 5′-pyrimidine residue and the C4 carbonyl group of a 3′-thymine, while CPDs are induced by the cycloaddition reaction of adjacent pyrimidine bases [1,2].

To protect the genome against these bulky DNA lesions, different molecular pathways were evolutionarily selected in eukaryotes, including DNA repair mechanisms such as nucleotide excision repair (NER) [1].

The NER pathway is divided into the transcription-coupled repair (TCR) and global genome repair (GGR) subpathways [1–4]. TCR is responsible for the repair of bulky DNA lesions from transcribed strands of active genes, whereas GGR is associated with the removal of DNA damage from transcribed strands of active genes, heterochromatin areas and non-active genes [1–4].

Although several proteins in complex are required to recognize bulky DNA lesions and promote NER, the xeroderma pigmentosum complementation group proteins (XPs) play critical roles. XPs include seven proteins termed XPA through XPG [1,4–6]. These proteins coordinate and promote the following basic steps in the NER pathway: (i) recognition of the distortion in the DNA molecule caused by a bulky nucleotide lesion, (ii) assembly of the repair machinery (the "preincision complex"), (iii) excision of the damaged nucleotide *via* dual incision, and (iv) synthesis of a new DNA fragment followed by ligation of the synthesized fragment with the pre-existing DNA molecule [6]. Additionally, XP proteins interact with a variety of proteins critical for genome organization and maintenance that are not directly involved in NER (reviewed in [4]).

Due to their critical role, mutations in XP proteins are commonly found in various cancer types and are responsible for a rare autosomal recessive disorder known as xeroderma pigmentosum (XP). Affected individuals exhibit a high predisposition for development of skin cancers and neurological degeneration, among other clinical symptoms; although not all XP patients exhibit the same effects [7]. Defects in NER are also related to Cockayne Syndrome (CS) and trichothiodystrophy (TTD), which are two conditions that lead to progeria [8]. The symptoms related to XP can be mild or severe, where severe affected XP patients may display combined phenotypes with CS or TTD diseases, sometimes leading to premature aging phenotype, developmental impairments and severe neurological abnormalities [9].

Considering the importance of XP proteins in NER and that their dysfunction results in XP disease, an understanding of the structure of XP proteins is necessary. However, few studies have examined the available information concerning the impact of mutations on XP proteins function and how post-translational modifications affect the function of these XP proteins.

Therefore, we aim to review the current knowledge of the structure and post-translational modifications of XP proteins, as well mutations that may affect their function and protein–protein interactions (PPIs). A review of the primary function of each XP is beyond the scope of this review, as this topic has previously been broadly reviewed (see [1,6]). In addition, we focus on the structure of XP proteins from XPA to XPG, not considering the XP variant DNA polymerase eta (XP-V).

2. The pathfinders: XPC and the UV–DDB complex

2.1. Mutations that affect XPC structure and critical regions

The first step of the GG-NER pathway is the recognition of bulky distortions in DNA promoted by the tight interaction between XPC and RAD23B. In general, XPC patients show no severe phenotypes, since they do not develop neurological abnormalities or exaggerated sunburn, but they can develop skin cancers [9]. However, a report of a male patient showing abnormal neuronal features and strong cell carcinomas and melanomas was reported by Khan et al. in 1998 [10].

Human XPC is a 940-residue DNA-binding protein that exhibits high affinity for 6-4 photoproducts and *N*-acetoxy-2-acetylaminofluorene adducts [11]. XPC forms a heterodimer with RAD23B, which is a multiubiquitin chain receptor, to recognize bulky DNA lesions in GG-NER [11]. It also forms a heterodimer with centrin 2, which is an essential protein for chromosome segregation and microtubule organization [12]. Centrin 2 is an important stabilizer of the XPC-RAD23B heterodimer and assists in XPC DNA binding [13,14] (Fig. 1; Table 1).

XPC is composed of a C-terminal region (residues 492–940) that contains the major PPI sites and the DNA-binding domain and an N-terminal region (residues 1–491) that contains an XPA-binding region (Fig. 1) [15]. To date, the first study to identify XPC protein structure was accessed using the XPC yeast orthologue Rad4 [16]. Rad4 was crystalized bound to a CPD lesion and the authors observed three 50–90 residues structurally related α/β domains characterized by a long β-hairpin (BHD1-BHD3) [16]. BHD1 is responsible for binding undamaged DNA, whereas BHD2 and BHD3 bind to the CPD lesion [16]. In humans, BHD1 corresponds to the regions 632–689, BHD2 to region 690–741 and BHD3 to region 742–833 (Fig. 1). The authors identified numerous residues that are responsible for Rad4 DNA-binding and are described in Table 1 and Fig. 1.

Site-directed mutagenesis of four conserved residues (Phe799Ala, Phe797Ala, Phe756Ala and Asp754Ala) in human XPC was used to evaluate DNA binding and DNA damage recognition [17] (Table 2). Nearly all of the heterologously expressed XPC mutants exhibited a reduced ability to bind undamaged DNA, with the exception of XPC (Phe799Ala), which retained DNA-binding properties [17]. These four mutants also exhibited decreased levels of accumulation at UV-damaged sites compared with wild-type (WT) XPC. FRAP measurements reveals that these mutations restricted XPC nuclear mobility and prevented the formation of stable recognition complexes [17].

XPC recognition of DNA damage is the first step of GG-NER and requires the subsequent recruitment the of the TFIIH complex to promote DNA denaturation and the assembly of the preincision complex [1,4,6]. Remarkably, the XPC (Phe797Ala) and XPC (Phe799Ala) mutants exhibited a decreased ability to recruit the TFIIH complex [17].

Another study utilized site-directed mutagenesis and DNA-binding assays to assess the role of different residues in XPC function [18]. The XPC (Phe733Ala), XPC (Trp690Ser) and XPC (Trp690Ala) mutants were unable to bind DNA lesions [18]. Other XPC mutations generated using site-directed mutagenesis did not affect the ability of XPC to bind DNA lesions. These authors also identified regions in XPC that play a critical role in the recognition of UV-damaged DNA regions by generating truncation mutants (Table 1; Fig. 1).

Based on the analysis of truncated XPC proteins, these authors also identified a 25-residue region (residues 742–766) that corresponds to a β-turn near BHD3 that appears to be crucial for XPC activity. Generation of truncation mutants ($XPC_{607–741}$ and $XPC_{607–766}$) confirmed that this region is necessary for XPC recruitment to UV-damaged sites [18]. Another study demonstrated that residues 606–742 constitute the primary region required for DNA binding, which is consistent with other findings that indicated that mutations inside and near this region lead to a loss in the ability of XPC to bind DNA [15]. Moreover, several other regions of XPC were proposed to be PPI sites [15] (Table 3 and Fig. 1).

Other mutations in the *XPC* gene were found in lung and colorectal cancer patients and in XP patients (Table 2; Fig. 1) [19–21]. Interestingly, a majority of the mutations found in XPC are located in the PPI and DNA-binding regions.